ROLE OF PITUITARY STALK IN WATER METABOLISM AND REGULATION OF ACTH-ADRENOCORTICAL SYSTEM

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FERENC A. LÁSZLÓ and KÁLMÁN KOVÁCS

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CONTENTS

INTRODUCTION

This monograph describes the investigations performed in rats submitted to pituitary stalk lesions. In the first place the water metabolism of animals bearing stalk lesions was analysed, but the morphology of the pituitary, furthermore the functional capacity of the ACTH-adrenocortical axis were also studied.

The majority of the experiments reported in this monograph have been published in different periodicals (László et al, 1961, 1962 a, b, c, d, 1963, 1964, 1965 a, b, 1966 a, b, c; Dávid et al, 1962, 1964, 1965, 1966; Kovács et al, 1962 a, b, c, d, e, 1963, 1964 a, b, 1965); however, some of the examinations are unpublished.

Our work has been supported also financially by the Scientific Council of the Hungarian Ministry of Health for some years. We should like to express our gratitude for this help. We are also very grateful to Professor M. Julesz who followed with attention our work, and gave us valuable advice. We are also very much obliged to our co-workers (Margit A. Dávid, L. Csernay, Julia Kocsis, Eva Horváth, Gizella Suták, S. Bertényi, O. Jenőfi). Without their devoted help this work could never have been completed. We should also like to thank Professor M. Földi, the chief editor of Studia Medica Szegediensia who accepted our work for publication, to Mrs. Marton for the translation, to Librarian Gy. Pálfy for the settlement of the affairs concerning the publication, furthermore to the Printing Company of Szeged for the printing.

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Chapter I

HISTORICAL REVIEW

The syndrome of diabetes insipidus is known since a long time. The main symptoms are polyuria and polydipsia. The specific gravity of the urine is low and the disorder of water metabolism reacts well to the administration of vasopressin. The essential character of the disease was first described by JOHANN PETER FRANK (1794), according to his concept diabetes insipidus can be characterised by: „a long lasting abnormally high secretion of urine not containing glucose, not caused by renal disease". At the end of the last century several papers were published dealing with the aetiology of the syndrome. OPPENHEIM (1896) mentioned lues, KAHLER (1886) the trauma of the skull, whereas KRUSE (1894) reported that all changes causing bitemporal haemianopsy can be associated with diabetes insipidus. Later based on numerous clinical observations (FRANK, 1910; FITZ, 1914; HALL, 1923; ROWNTREE, 1924; FINK, 1928; FUTCHER, 1931) it was suggested that this syndrome develops following impairment of the hypothalamus-pituitary region. ERDHEIM in his study published in 1904 gave its detailed pathomorphological analysis and correlated the neurohypophysis with diabetes insipidus.

In addition to the clinical observations data relating to the results of animal experiments also appeared. CLAUDE BERNARD (1849) induced by pricking the fourth ventricle of the brain a state associated with glycosuria and polyuria, however, in some animals only the urinary output increased without the excretion of glucose.

ECKHARD (1872) could induce polyuria by impairment of the pons and cerebellum. Apart from these observations experiments studying the effect of whole pituitary and posterior pituitary extracts supplemented the knowledge on this subject. OLIVER and SCHÄFER (1895) attributed a blood pressure elevating effect to the pituitary extract. HOWELL demonstrated three years later (1898) that the neurohypophysis was responsible for this effect. DALE (1909) reported that neurohypophysial extracts caused contraction of the uterus. BELL (1909) described the role of the neural lobe played in the stimulation of the peristalsis of the intestines and OTT and SCOTT (1910) its part in the secretion of milk. The experiments of MAGNUS and SCHÄFER (1901) and SCHÄFER and HERING (1906) dealing with the effect of the neural lobe on the water metabolism were interesting. They found that the posterior pituitary contains a diuretic substance. The misleading conclusions were obviously due to the fact that these investigators performed their examinations on anaesthetized animals. This false assumption dominated for years in the literature till VON DEN VALDEN (1913) and inde-

pendently of him FARINI (1913) established that in humans the neurohypophysial extract diminishes urinary output. However, decades had to elapse after the description of these adequate observations for the conditions to develop enabling the isolation of the antidiuretic hormone. DE VIGNEAUD and his co-workers (1954) reported the exact structure of the two hormones of the posterior lobe. It should be remarked that among the pituitary hormones the structure of oxytocin and vasopressin was determined first and their synthetic production also succeeded (DE VIGNEAUD et al. 1954). Subsequently many active analogues were synthetized (BOISSONNAS et al. 1956).

Although CAJAL already demonstrated in 1894 that there exists a direct neural connection between the hypothalamus and the neurohypophysis and described the presence of the supraoptico-hypophysial tract in two days old mice, the systematic research work concerning the function of this tract playing such an important role in the regulation of the posterior lobe only started about 50 years ago. The introduction of a new technique to injure the brain created adequate conditions to perform the investigations. The new method rendered possible to damage precisely well-defined portions of the brain (HORSLEY—CLARKE, 1908). Using this technique CAMUS and ROUSSY (1920) observed diabetes insipidus in dogs following lesion of the hypothalamus. One year later working with a similar method BAILEY and BREMER (1921) succeeded in inducing a polyuric state in dogs. Subsequently PINES (1925) and GREVING (1926) pointed out the importance of the hypothalamo-neurohypophysial neural connections, i. e. that of the supraoptico-hypophysial tract. RICHTER was the first (1938) to observe that a purely neural lesion causes persistent polyuria in rats. Somewhat later FISHER et al (1938) studied in detail the morphology and function of the hypothalamo-neurohypophysial system in cats. In their work which now may be considered as a fundamental one they suggested that the hypothalamus and posterior pituitary compose a functional entity. According to their concept the system consists of three parts: 1. the two magnocellular nuclei located in the anterior portion of the diencephalon, the supraoptic and the paraventricular nuclei. 2. These nerve centres are connected by the supraoptico-hypophysial tract with the posterior pituitary although a few fibres also originate from the nuclei of the tuber cinereum. 3. The median eminence, the stalk and mainly the posterior lobe of the pituitary can be considered as the recipients of the nerve fibres. The authors reported that an impairment of any portion of the system may elicit diabetes insipidus which can be influenced by the administration of a neurohypophysial extract. Later the same group of investigators (MAGOUN et al. 1939) succeeded in confirming the above conclusions in monkeys. The concept of the importance of the neural connection is also supported by the experiments in which a reduction of the urinary output was attained by means of electric stimulation of the supraoptico-hypophysial tract (HARRIS, 1947).

The studies of VERNEY (1946, 1947) on the other hand, elucidated the problem of physiological stimulation. It was established that hypertonic NaCl administered into the internal carotid artery via the osmoreceptors enhanced ADH secretion. Later, some authors (HARE, 1947, AIMES et al.

1950; FRIEDMANN et al, 1956) also emphasized the importance of the osmotic factors thus confirming the results of VERNEY.

Hence, the question arose: which part of the system outlined above produces the antidiuretic hormone? For a long time the most widely accepted view was that the pituicytes of the posterior lobe produce the adiuretin and oxytocin (GERSH, 1939). However, since the neurohypophysis consists of a neural and not of a glandular structure it has been assumed that the hormones of the posterior pituitary are produced somewhere else. Thus HERRING (1908, 1914) suggested that the pars intermedia was responsible for the formation of the effective substances of the posterior pituitary. In his opinion it reaches the neural lobe by diffusion and through the pituitary stalk it reaches also the brain. Other data pointed towards the hypothalamus. POPPI (1930) assumed that the diencephalon has a secretory function. Previously others (TRENDELENBURG, 1928; SATO, 1928) showed that in the hypothalamus an activity corresponding to that of the neurohypophysial hormones could be found. Subsequently MELVILLE and HARE (1945) reported that the antidiuretic activity of the hypothalamus is connected with the supraoptic region and that following the lesion of the pituitary stalk its effective substance decreases owing to the retrograde degeneration of the supraoptic nucleus. Extracts of the brain portions situated far from the supraoptic nucleus and the supraoptico-hypophysial tract did not show oxytocin, vasopressin or antidiuretic activity which could have been measured by biological methods (HILD, ZETLER, 1951, 1952a).

New perspectives opened up to the investigators after the discovery of neurosecretion. SCHARRER and GAUPP (1933) were the first to report that in humans in the cytoplasm of the supraoptic and paraventricular nuclei colloid droplets can be seen which may play a role in water metabolism. SCHARRER and SCHARRER (1940, 1945) described subsequently similar observations in bone fishes. Some years later different staining procedures were elaborated to make these so-called Gomori positive colloid, particles, the neurosecretory material detectable (BARGMANN, 1949; DAWSON, 1953; GABE, 1953; SLOPER, 1955). A further problem was to elucidate the transport of this substance. BARGMANN and SCHARRER (1951) observed that in frogs in the proximal portion of the severed pituitary stalk the neurosecretory material accumulated within a few days. Thus, they assumed that the colloid droplets reached the neural lobe from the diencephalon. In his paper MÜLLER (1955) dealt with a case in which a meningeoma compressed the infundibulum and proximally to it an accumulation of neurosecretory material could be detected. SLOPER and ADAMS (1955) observed a similar phenomenon in hypophysectomized humans operated because of a disseminated cancer. The outstanding work of HILD (1954) should be mentioned in which he reported that in tissue cultures the colloidal granules migrate along the axons of the nerve cells distally. Recently BACHRACH and KORDON (1960) have furnished evidence about the proximodistal transport of the neurosecretory material.

Thus, the view has generally been accepted that the neurosecretory material exerting antidiuretic activity is produced by the magnocellular nuclei of the hypothalamus. It migrates from these ganglion cells along the

supraoptico-hypophysial tract distally to the posterior lobe, where the colloid granules are stored and secreted on suitable stimuli as ADH into the circulation (SCHARRER and SCHARRER, 1940, 1954; PALAY, 1953). This theory is described in detail in Bargmann's monograph (1954).

Does, however, the neurosecretory material correspond to the active hormone? Important facts support this view. Thus, the content of the neurosecretory material changes parallel with the functional state of the neurohypophysis. As a result of dehydration the colloid droplets disappear from the posterior lobe and following rehydration they accumulate again (ORTMANN, 1951; LEVEQUE and SCHARRER, 1953; KOVÁCS et al 1954; BACHRACH, 1958). Simultaneously after water deprivation ADH secretion increases (GILMANN and GOODMAN, 1937) and the adiuretin content of the hypothalamus and posterior pituitary decreases (KOVÁCS, 1957). HILD and ZETLER (1952b) studied the correlation of the amounts of ADH and neurosecretory material in the diencephalon of dogs, pigs and humans. They established that the amount of the Gomori positive substance and that of the hormone is higher in dogs than in humans, however, in both cases the amounts of the two substances corresponded to each other. HILD and ZETLER (1951, 1952a) found that the portions of the hypothalamus which do not contain neurosecretory material, do not exert an antidiuretic activity. Others (BARGMANN and SCHARRER, 1951) could not demonstrate neurosecretory granules in the posterior lobe from which vasopressin was already extracted It was also observed that the cystine content of both the hormone and the neurosecretory material is very high (BARRNETT and SELIGMAN, 1954; SLOPER, 1955).

Since their papers were published many others have appeared on this subject (BENOIT and ASSENMACHER, 1953; RODECK, 1957; SLOPER, 1958; RINNE, 1960; MOLL and DE WIED, 1961, 1962; GABE, 1966). The solution of the above mentioned problems will only be possible when the direct chemical determination of the hormones contained in the neurosecretory material will be clarified.

After the discussion of the functional unity of the hypothalamus and posterior lobe of the pituitary gland it is obvious that the pituitary stalk as the morphological link connecting the two parts plays an important role in the water metabolism of the organism. Owing to the different kinds of operations, the differences in the techniques used (injury of the adjacent areas) and the various methods applied at the examinations of the water metabolism the conclusions drawn from experiments and clinical observations did not furnish unequivocal results.

PAULESCO (1908) performed stalk lesion in dogs, however, he did not mention a disturbance of the water metabolism. Controversely, two years later CUSHING and GOETSCH (1910) observed a pronounced polyuria in an animal after having ligated with a silver clamp and destroyed its pituitary stalk. Other authors (KARPLUS and KREIDL, 1910; MORAWSKI, 1911) reported that in monkeys diabetes insipidus did not develop following stalk lesion. CROWE et al. (1910) were the first to draw attention to the role of the anterior pituitary in water metabolism as after hypophysectomy they could not observe increased urinary output. However, following transplantation of the anterior pituitary the animals became polyuric; this phenomenon

ceased after the pituitary had again been removed. In humans the first stalk lesion was performed by Cushing — the stalk was ruptured by chance at a pituitary biopsy — but polyuria was not mentioned in the description of the case (DANDY and GOETSCH, 1911); it is true that the patient died in the early postoperative period. BELL (1919) transected the stalk in two dogs, but a substantial change did not occur in the water metabolism of the animals. On the other hand, when a platinum plate was inserted between the two stumps of the stalk a significant polyuria ensued (DOTT, 1923). According to the experiments of MAHONEY and SHEEHAN (1936) the conclusion can be drawn that the different animal species do not respond in the same way. These authors performed the stalk lesion means of silver clamps and whilst in dogs diabetes insipidus developed, in monkeys the urinary output did not increase. It should be noted that in the first case the hypothalamus was also impaired. Subsequently DANDY (1940) reported a case in which the patient with a stalk lesion suffered from diabetes insipidus for 11 years after the operation. The investigations of RASMUSSEN (1938, 1940) represented a significant advance showing that after stalk lesion owing to retrograde degeneration the number of the ganglion cells of the supraoptic nucleus diminished. The experiments of FISHER et al (1938) are also important. They induced in cats by bilateral lesions of the hypothalamus a characteristic syndrome associated with polyuria. Diabetes insipidus could only be observed in animals in which both supraoptic nuclei and the unmyelinated nerve fibres emerging from them were completely destroyed. The rupture of the nerve tracts connecting the two poles of the adiuretin system brought about characteristic changes: 1. as a result of the retrograde degeneration proximally to the lesion the number of the nerve fibres decreased, in the supraoptic nucleus many ganglion cells disappeared and the remaining ones got smaller, 2. distally from the destruction the changes were still more striking and extensive atrophy of the posterior pituitary could be seen. O'CONNOR (1951) confirmed the above results in dogs and established that by removal of the posterior pituitary polyuria could also be induced, however, the increase of urinary output was more pronounced following stalk lesion. Recently RANDALL et al. (1957, 1960) dealt with the problem in humans. The review of SHARKEY et al. (1961) should also be mentioned. They drew the conclusion that following lesion of the pituitary stalk in humans all parts of the supraoptico-neurohypophysial system become indeed damaged and the severity of the diabetes insipidus depends upon the extent of the injury. In addition, the authors found a difference between stalk lesions placed in a lower or higher region; according to their observations in the latter case polyuria was more extensive.

It is beyond doubt that these detailed analyses promoted the better understanding of the problems, however, many questions concerning the role of the stalk are still unsolved. The conclusion is rendered more difficult because according to some data besides the ADH system described a centre regulating the water intake can also be found in the hypothalamus. The stimulation of this centre causes an increase of the water intake (ANDERSSON, 1953; ANDERSSON and MCCANN, 1955; GREER, 1955), whereas its focal injury induces oligodipsia (GALE et al. 1961).

Furthermore, it is known that following lesion of the stalk a disturbance

in the function of the anterior pituitary also ensues (HARRIS, 1950, 1955b; BARRNETT and GREEP, 1951; FORTIER, 1951; DONOVAN and HARRIS, 1954; HALÁSZ et al. 1963; KENNEDY and PARKER, 1964) which may also influence the development of the disturbance of the water metabolism.

The pituitary is connected with the hypothalamus by the pituitary stalk. Currently the view that the function of the pituitary is regulated by the diencephalon is generally accepted (HARRIS, 1955a; JULESZ, 1957; LISSÁK and ENDRŐCZY, 7960; DONHOFFER, 1961; SZENTÁGOTHAI et al. 1962). The destruction of the stalk deleteriously alters the function of this regulating mechanism; the blood supply of the anterior pituitary decreases and ischaemic necrosis develops in the anterior lobe. (UOTILA, 1939; BARRNETT and GREEP, 1951; GREEP and BARRNETT, 1951; DANIEL and PRICHARD, 1956, 1957 a, b, c, 1958; RUSSELL, 1956; HOLMES, 1961, 1962; GODMAN et al, 1962; ADAMS et al, 1963, 1964; KENNEDY and PARKER, 1964). The venous blood flow is also reduced over shorter or longer periods (HARRIS, 1955b).

The arterial blood supply of the pituitary is independent of the brain, whereas its venous network is in close connection with the capillaries of the diencephalon. This phenomenon has already been described in the XVIIIth century by LIEUTAUD (1742). POPA and FIELDING 200 years later (1930, 1933) discovered it again and termed this vascular system: „pituitary portal vascular system in the regulation of the function of the adonohypo- emphasized by several other authors (GREEN and HARRIS, 1947; GREEN, 1951; WINGSTRAND, 1951). No unequivocal conclusion could be drawn concerning the direction of blood flow in the portal vessels. POPA and FIELDING (1930, 1933) believed that the blood flows from the pituitary towards the diencephalon. This view was in harmony with the assumption of CUSHING (1933) who suggested that the hormones of the posterior pituitary reach the hypothalamus via the veins of the stalk and they exert an important effect on the diencephalic structures. This theory contradicts the opinion of WISLOCKI and KING (1935). According to their observations the blood flows in an opposite direction, i.e. from the hypothalamus into the anterior pituitary. HOUSSAY et al. (1935) and GREEN (1947) examined directly the circulation in the portal vessels on anaesthetized amphibia and established that the blood flows from the median eminence towards the pars distalis. GREEN and HARRIS (1949) confirmed the above observations on living rats. Undoubtedly the majority of the investigators hold the view that the portal circulation transports the blood from the capillaries being in contact with the neural elements of the hypothalamus to the capillary system of the adenohypophysis, however, according to some observations the possibility cannot be excluded that under certain conditions the direction of the blood flow may reverse in the living organism. TÖRÖK (1954) injected dye into the carotid artery of anaesthetized dogs and found that in the vessels located at the periphery of the pituitary stalk the blood flows towards the pars distalis, however, in some smaller vessels of the upper part of the stalk which are in contact with the diencephalon the blood circulates in the opposite direction. This observation was later confirmed by SZENTÁGOTHAI et al. (1957).

The functional significance of the portal circulation is an important problem. According to the theory of SPATZ (1951) and NOWAKOWSKI (1955) the

capillary loops located in the median eminence contain chemoreceptors. These chemoreceptors are stimulated by the hormone content of the blood and may bring a descendent neural function into action. This view owing to the absence of a direct neural connection between the hypothalamus and adenohypophysis has lost its actuality.

Lately the role of chemical substances, the so-called mediators or releasing factors transported by the portal vessels from the hypothalamus to the anterior pituitary has come into the forground of interest. Previously the existence of universal mediators was assumed which were supposed to stimulate the hormone production of the whole anterior lobe. According to MARKEE et al. (1948) and SAWYER et al. (1950) the adrenergic substances, to BENOIT and ASSENMACHER (1953), PALAY (1953) and ROTHBALLER (1953) the neurosecretory material, to MIRSKY et al. (1954) the hormones of the posterior lobe, to SLUSHER and ROBERT (1954) hypothalamic lipids and lipoproteins, to HARRIS et al. (1952) histamine are responsible for the regulation of the function of the pars distalis. Recently it has been established that the adrenergic and cholinergic substances exert only a quantitative effect on the portal circulation, i.e. they alter the amount of the blood flowing through the portal vascular bed (WORTHINGTON, 1960). At present it is generally accepted that the single anterior pituitary hormones are stimulated by special substances, the so-called releasing factors. Thus, the secretion of ACTH is stimulated by the corticotropin releasing factor (CRF) SAFFRAN et al. 1955; SCHALLY and GUILLEMIN, 1960, 1963; DE WIED, 1961; VERNIKOS-DANELLIS, 1965). In recent years the releasing factors of the other adenohypophysial hormones, furthermore the prolactin secretion inhibiting factor (PIF) have also been detected (SZENTÁGOTHAI et al. 1962; JULESZ and KOVÁCS, 1966).

According to the data described above the destruction of the pituitary stalk seemed to be a suitable method to demonstrate the importance of the portal vascular system in the regulation of the function of the adenohypophysis. Using this procedure various investigators have drawn different conclusions. HARRIS (1950, 1955b) and DONOVAN and HARRIS (1954) found a close correlation between the portal vasculature and adenohypophysial function. THOMSON and ZUCKERMAN (1955) on the other hand supposed that the anterior lobe deprived of the portal vessels is capable of secreting hormones. The possible role of the infundibular recess as a component between the hypothalamus and the pars distalis, must be taken into account here, too (LÖFGREN, 1960).

An interesting and not completely solved problem is the connection between adenohypophysial function and diabetes insipidus.

There are only a few data in the literature suggesting that following hypophysectomy diabetes insipidus develops (LIPSETT et al. 1955; IKKOSS et al. 1955). Presumably in these cases the removal of the pituitary was not complete and the stalk was injured. The majority of the investigators reported that total hypophysectomy does not cause permanent polyuria (CAMUS and ROUSSY, 1920; FEE, 1929; PENCHARZ et al. 1936; LEAF et al. 1952; LUFT et al. 1955; BRUNNER et al. 1956; GILLMAN and GILBERT, 1956).

HANN (1918) was the first to analyse this problem in detail. In her fundamental clinico-pathological study she stated that long-lasting diabetes in-

sipidus only occurs when the anterior pituitary is intact. Her conclusions have also been confirmed by other authors (RICHTER, 1934; KELLER et al. 1936; FSICHER et al. 1938; KELLER 1937). KELLER (1937) and SCHWEIZER et al. (1949) could elicit polyuria by administration of an adenohypophysial extract of the hypophysectomized animals. Some investigators (HEINBECKER and WHITE, 1941; LIPSETT et al. 1956, 1957) on the other hand assumed that the extent of the polyuria depends less on the damage of the anterior pituitary than on the disturbance in the function of the supraoptico-neurohypophysial system. Conversely, EHNI and ECKLES (1959) observed in patients suffering from cancer of the breast in whom the stalk had been dissected that a strictly inverse correlation exists between the severity of the diabetes insipidus and that of the extent of anterior pituitary necrosis due to the operation. This may be the reason why MAHONEY and SHEEHAN (1936) did not observe polyuria in monkeys following destruction of the stalk, but later HOLMES (1963) found a substantial difference in the water metabolism of monkeys with stalk lesions depending on the impairment of the pars distalis. In the cases in which a plate was inserted between the two ends of the pituitary stalk and a marked hypofunction of the anterior lobe ensued polyuria did not develop. In the absence of the separating plate if the function of the anterior pituitary was normal significant diabetes insipidus occurred.

According to some data somatotrop hormone (STH) may be responsible for the diuretic function of the anterior lobe (WHITE et al. 1949, 1951), whereas others attribute this effect to the adrenocorticotrop (ACTH) and the thyreotrop hormones (TSH) (PICKFORD and RITCHIE, 1945; HEINBECKER et al. 1947). The role of TSH was also emphasized by MAHONEY and SHEEHAN (1935) and FINDLEY (1937). They demonstrated that after thyreodectomy the severity of diabetes insipidus decreases. The importance of the thyroid gland is also supported by the experiments in which the diuretic effect of thyroid extracts has been demonstrated (MOSONYI and MATSCH, 1955). Recent examinations, however, suggest that neither the STH nor the thyroid homones enhance urinary output (GALE et al. 1961).

The most generally accepted theory is that the anterior pituitary exerts its diuretic effect via the ACTH-adrenocortical axis. It is known since a long time that relating to the water metabolism there is an antagonism between the ADH and ACTH systems (SILVETTE and BRITTON, 1938; COREY and BRITTON, 1941; ANDERSON and MURLIN, 1942). Following adrenalectomy the extent of diabetes insipidus diminishes (GAUNT et al. 1949; CHESTER JONES, 1957; KENNEDY and CRAWFORD, 1961). In two cases of Sheehan syndrome owing to the simultaneous impairment of the anterior and posterior lobes DINGMAN et al. (1958) could observe the manifestation of diabetes insipidus only after cortisone treatment. SHARKEY et al. (1961) stated that after stalk lesion polyuria only ensues if in addition to the damage of the supraoptico-hypophysial tract there remain many intact cells producing ACTH in the anterior pituitary or if the patient is given cortisone substitution. One of us (KOVÁCS, 1963) analysed this question in detail and established that ACTH is responsible for the diuretic function of the pars distalis. The secretion of ACTH is regulated by the hypothalamus. Presumably ACTH exerts its action via the adrenocortical hormones

by altering renal blood flow. Following hypophysectomy renal blood flow decreases (WHITE et al. 1942) and the glomerular filtration diminishes (WHITE and HEINBECKER, 1938; WHITE et al. 1941; PICKFORD and RITCHIE, 1945; PICKFORD and WATT, 1950; Boss et al. 1952; HOWELL et al. 1955; HEIDENREICH et al. 1961). Administration of cortisone to hypophysectomized rats moderates the decrease of renal blood flow (KOVÁCS et al. 1965). Other authors have reported that following adrenalectomy (ROBERT and PITTS, 1952) and in the case of adrenocortical insufficiency (BURSTON and GARROD, 1952) glomerular filtration decreases, as well.

The examination of the effect exerted by the hypothalamus on the water metabolism is not an easy task. To study this question three main methods — pituitary transplantation in hypophysectomized animals, neurohypophysectomy and destruction of the pituitary stalk — are available; all these interventions disrupt the connection between the hypothalamus and the pituitary gland. The removal of the pituitary and grafting of the anterior lobe distant from the hypothalamus markedly alters the water metabolism. Following hypophysectomy after a polyuric period of 1—2 days as a result of the lack of the anterior lobe, water retention can be observed. Transplantation of the pituitary on a site distant from the hypothalamus and hypophysial stalk does not abolish this state as the ACTH producing activity of the pituitary graft is minimal (KOVÁCS, 1963). The investigation of the disturbance of water metabolism in neurohypophysectomized animals has also disadvantages, because following hypophysectomy the proximal stump of the stalk regenerates and a posterior pituitary-like structure develops (STUTINSKY, 1951; BILLENSTIEN and LEVEQUE, 1955) possessing a certain functional capacity (MOLL and DE WIED, 1962). The destruction of the hypophysial stalk is far more suitable for the examination of the function of the hypothalamus and posterior pituitary concerning water metabolism. After this operation the damage of the adiuretin system is the most pronounced, the reorganization of the nervous tissue of the stalk cannot be seen and the disturbance of the water metabolism is the most striking. It is beyond doubt, however, that for the examination of the problem the following factors cannot be neglected: the state of the portal circulation, the extent of the ischaemic necrosis, as well as the volume of the surviving anterior lobe. It must be emphasized that the study of the hypothalamus playing such an important role in the regulation of the water metabolism can only be performed in a complex manner, by taking all these factors into account.

Chapter II.

MORPHOLOGICAL AND CIRCULATORY CHANGES IN THE HYPOTHALAMUS-HYPOPHYSIAL SYSTEM AFTER LESION OF THE STALK

1. Location of the lesion. Histological alterations and changes of the volume of the pituitary

The experiments were carried out on white rats of both sexes weighing 180—200 g fed on a standard diet. The destruction of the pituitary stalk was performed under nembutal anaesthesia (dose: 4,0 mg/100 g body weight ip) by means of a Horsley—Clarke apparatus (Horsley and Clarke, 1908). The lesion was produced by electrocautery with a metallic filament electrode isolated with a thin glass tube the diameter of which was 0,1 mm and its free end 0,6 mm. The intensity of current was 5 m A and the time of manipulation 8 sec.

At various intervals after stalk lesion the animals were decapitated and autopsied. The brain was removed together with the pituitary to render possible a thourough examination of the stalk. After fixation in Susa's solution the region of the hypothalamus connected with the pituitary and stalk was embedded in paraffin. Serial sections were made in the frontal plane 10 microns thick at 100 microns level. The sections were stained with haematoxylin-eosin. In some cases special staining methods were applied. The results will be reported later. The location and extent of the lesion was precisely determined. On the drawing made in the sagittal plane of the hypothalamus the typical lesion is denoted by a circle (Fig. 1.). The

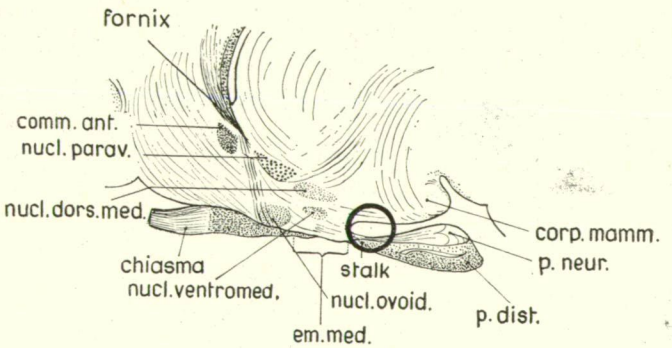


Fig. 1. Sketch of the hypothalamus in the sagittal plane. The site of the typical lesion is denoted by a circle

greater part of the destroyed area is located in the praemamillar region. But in many cases it touches the posterior part of the median eminence and the anterior one of the mamillary nuclear group. The stalk lesion was considered to be complete in rats in which both the proximal and

distal portions of the stalk were destroyed in the whole cross section (Fig. 2.).

The determination of the pituitary volume was only carried out in animals in which, according to the histological studies, the operation had been

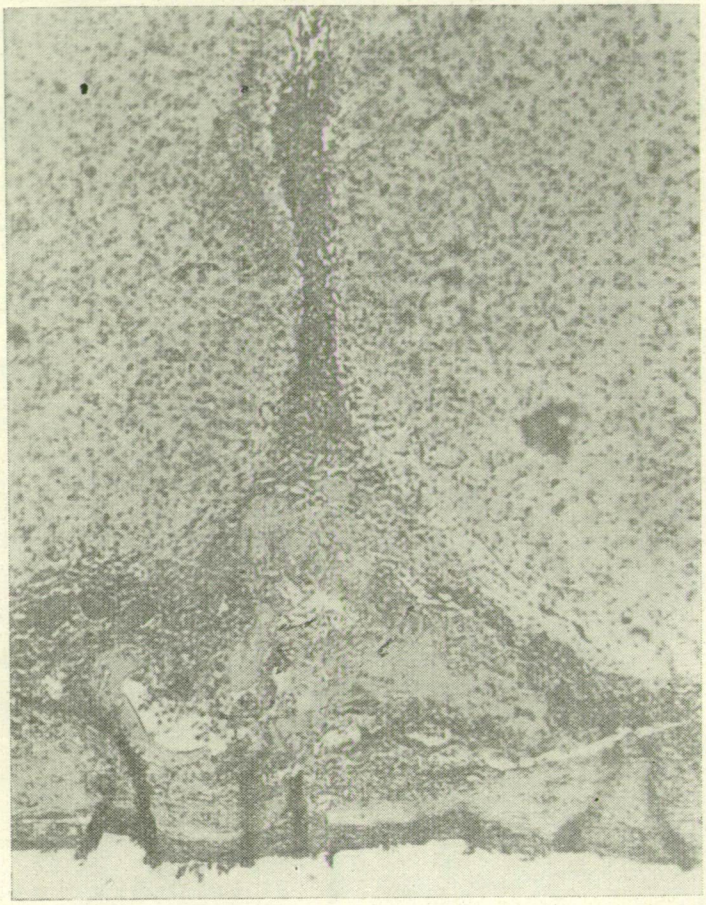


Fig. 2. The hypothalamus of a rat with total stalk lesion operated 2 days previously. In the premamillary region a crater shaped destroyed area can be seen. Haem. —Eo. staining, 88 X.

adequate. The serial sections mentioned above were used for the determination of the pituitary volume. The corresponding area of each section was projected in 60 X magnification by means of a magnifying apparatus and a drawing was prepared. The size of the magnified picture of the pituitary areas obtained in this manner was determined by means of a planimeter. From these values the real size of the area examined was calculated and multiplied by the distance between the single sections (100 microns) and finally these values were summed up. Expressed as a formula:

$$v = \frac{m}{e^2} (a_1 + a_2 + a_3 \ldots\ldots + a_n)$$

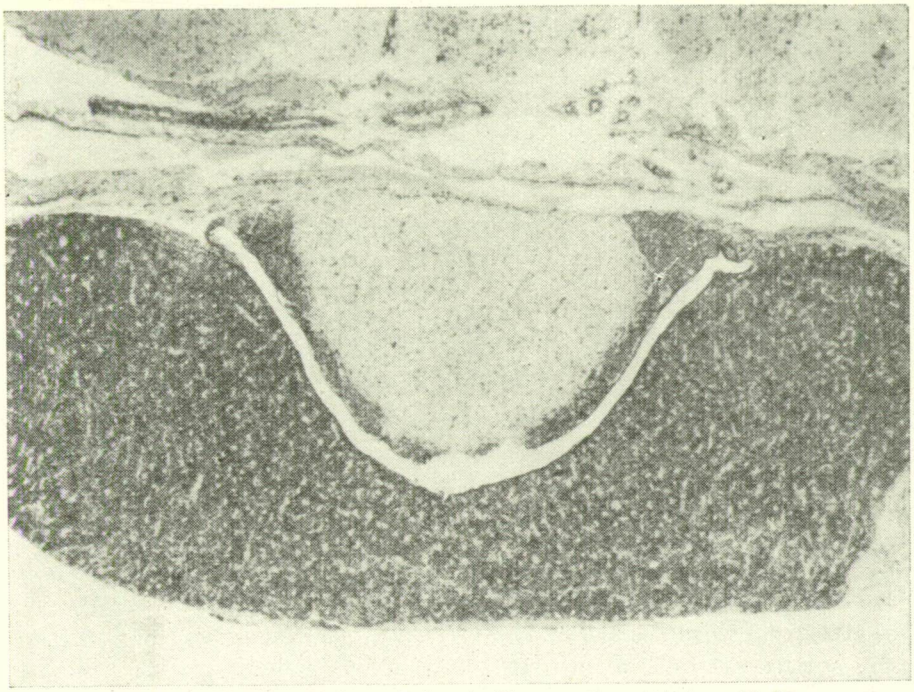


Fig. 3. Pituitary of control rat. Haem.—Eo. staining. 35 X.

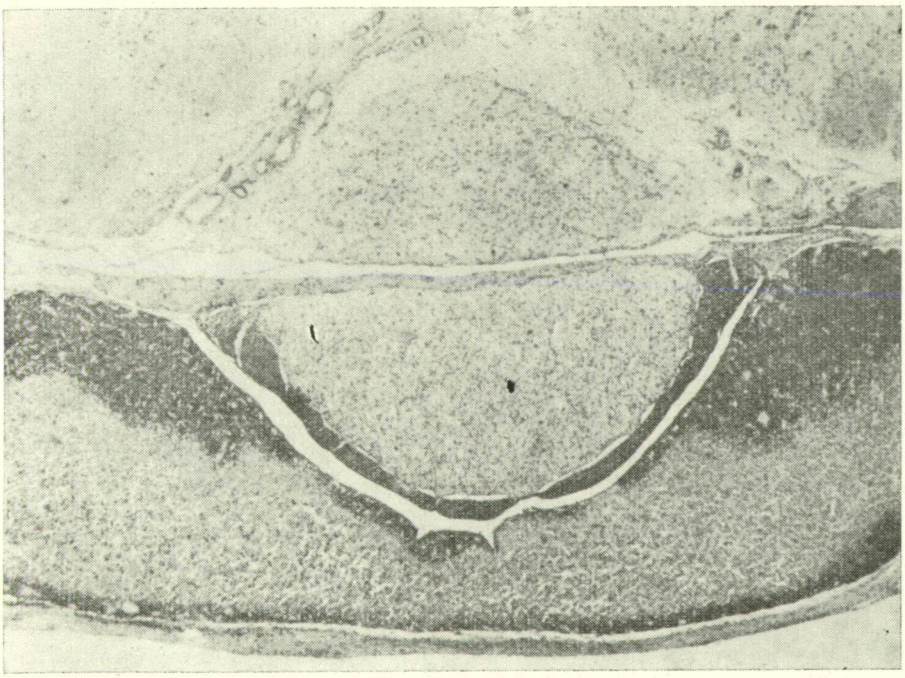


Fig. 4. Pituitary of a rat with stalk lesion performed 2 days previously. The central necrotic part of the pars distalis is surrounded by a surviving rim. Haem.—Eo. staining, 35 X.

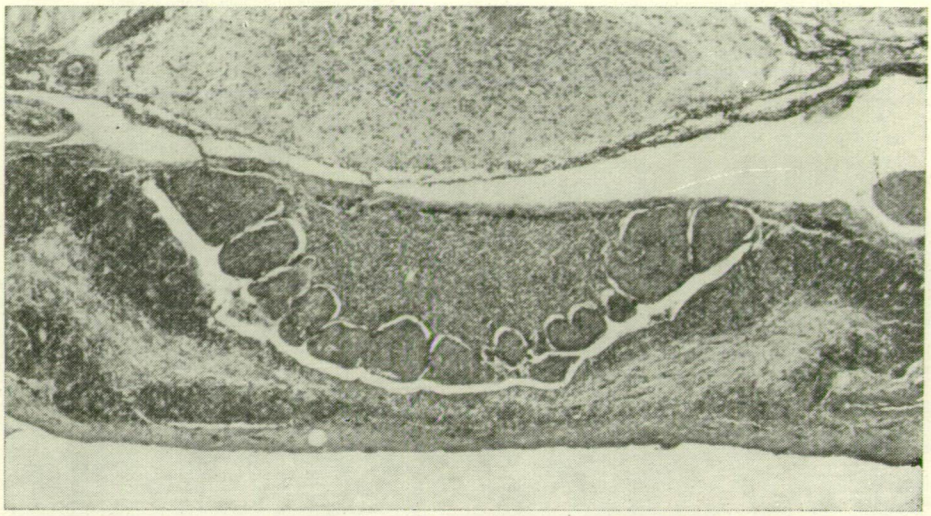


Fig. 5. Pituitary of a rat with stalk lesion performed 8 days previously. In the centre of the pars distalis fibrosis can be observed. The pars nervosa in atrophied. Haem.—Eo. staining, 35 X.

where V=volume expressed in mm^3, m=real height of sections (0,1 mm), e^2=the square of the linear magnification (60^2=3600), $a_1+a_2+a_3 \ldots a_n$ = =area of the magnified pituitary areas expressed in mm^2.

The results were also evaluated biometrically by Student's „t" test.

On analysing the histological alterations following destruction of the stalk 1—2 days after the surgical manipulation characteristic changes were found in the anterior pituitary. One day after the operation typical ischaemic necrosis could be observed in the central part of the anterior lobe; cell boundaries were blurred, the cell nuclei showed signs of pyknosis, rhexis and lysis. Two days following the operation relatively homogeneous eosinophil necrosis could be observed. The histological picture resembled that found by SHEEHAN (1937), SHEEHAN and STANFIELD (1961) in humans suffering from postpartum pituitary necrosis. The intact parenchyma only remained as a narrow rim. After 6—8 days connective tissue formation started. Some weeks after the operation (3—4 weeks) necrosis could no longer be found, instead of it in the middle of the anterior lobe a shrinking fibrotic scar consisting of cell poor connective tissue was seen.

In the pars intermedia no essential changes could be observed. In a few rats its structure was slightly oedematously loosened. In the animals in which the stalk had been destroyed some weeks earlier the pars intermedia seemed broader.

Immediately after the operation no appreciable changes could be found in the neural lobe. Later it gradually atrophied and seemed to contain a more abundant amount of cells; the cell nuclei were more densely located. The observed alterations are presented in Figs. 3—6.

The quantitative changes are shown in Table 1. It can be seen that the whole volume of the pituitary becomes gradually smaller, this is most pronounced in the animals operated some weeks previously. In the anterior pituitary (pars distalis) of the animals operated 1—2 days previously the

Table 1.

Hypophysial volume in rats with stalk lesion

Group		Time elapsed after operation	No of animals	Total hypophysis mm³	Pars distalis			Pars intermedia mm³	Pars nervosa mm³
					total mm³	destroyed mm³	intact mm³		
I.	Intact	—	12	6,989 ±0,336*	5,200 ±0,226	—	5,200 ±0,226	0,471 ±0,023	1,319 ±0,126
II.	Stalk lesion	1—2 days	12	6,579 ±0,403	5,166 ±0,394	2,672 ±0,295	2,493 ±0,261	0,404 ±0,027	1,009 ±0,101
III.	Stalk lesion	8 days	12	3,587 ±0,156	0,623 ±0,129	0,894 ±0,054	1,729 ±0,151	0,511 ±0,042	0,453 ±0,050
IV.	Stalk lesion	More than a month	12	2,785 ±0,141	1,949 ±0,096	0,309 ±0,028	1,715 ±0,132	0,556 ±0,043	0,279 ±0,033
			I/II.	$p>0,05$	$p>0,05$	—	$p\ll0,001$	$p>0,05$	$p>0,05$
Probability:			I/III.	$p\ll0,001$	$p\ll0,001$	—	$p\ll0,001$	$p>0,05$	$p<0,001$
			I/IV.	$p\ll0,001$	$p\ll0,001$	—	$p\ll0,001$	$p>0,05$	$p\ll0,001$

* Standard error

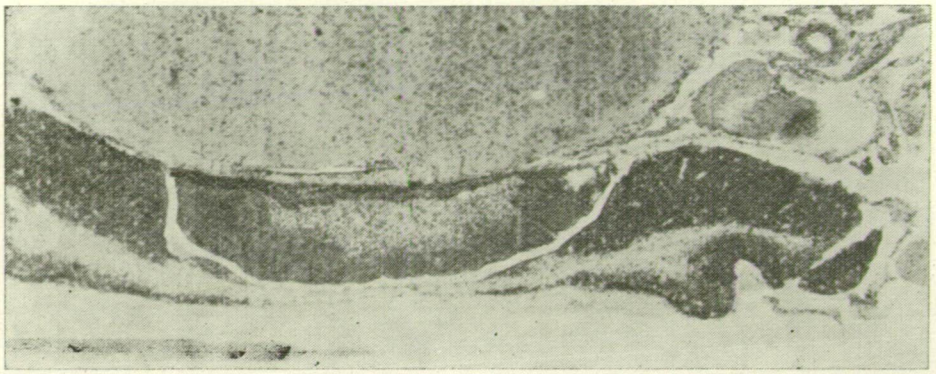


Fig. 6. Pituitary of rat with stalk lesion performed more than a month previously. In the centre of the pars distalis a tiny fibrotic area is visible. The pars nervosa is considerably atrophied. Haem.—Eo. staining, 35 X.

necrotic area was very extensive, it exceeded the size of half of the anterior lobe. The part which remained alive was the largest in the animals operated 1—2 days before. In the rats operated on 8 days previously it was somewhat smaller, later its volume did not change significantly. A broadening of the alive peripheral rim could not be observed in any case.

In the pars intermedia this decrease in size did not take place. We felt that in some cases the volume of the intermediate lobe was greater, this may also be seen in the Table in the mean values, but the alterations were not significant.

A considerable gradual atrophy of the neural lobe could be observed.

For the sake of comparison we found it necessary to present the changes on a percentage basis in a separate Table (Table 2). It may be seen that as compared to the total pituitary there is no significant change in the percentile volume of the adenohypophysis, although a small extent of decreasing tendency may be observed. On a percentage basis the pars intermedia markedly increased in size, hence the hypertrophy could be considered relative. In the neural lobe the atrophy was also in this respect uniform and gradual.

The extent of the change in the volume of the necrosis of the anterior pituitary expressed in per cents could have caused misleading conclusions to have been drawn. Namely, besides the gradual decrease of the necrotic part, the volume of the intact anterior pituitary showed a percentile increase. This alteration was, however, only virtual as it can be seen from the previous table that the total adenohypophysial volume did not increase significantly in an absolute sence, it even decreased. Real hypertrophy did not occur, as the disappearance of the extensive necrosis following the destruction of the stalk was not due to the hypertrophy of the intact rim, but to the fibrotic atrophy of the necrotic area.

Our investigations showed that following the lesion of the stalk characteristic alterations took place in the pituitary of the rats. The literature contains numerous clinical and experimental data on this subject. The observations suggest, however, that, following destruction of the stalk elicited with various operative techniques on different species, no unequivocal al-

Table 2.

Hypophysial volume in per cent in rats with stalk lesion

Group		Time elapsed after operation	No of animals	Referring to the whole hypophisis			Referring to the whole pars distalis	
				pars distalis %	pars intermedia %	pars nervosa %	destroyed pars distalis %	intact pars distalis %
I.	Intact	—	12	74,6 ±1,0*	6,9 ±0,3	18,5 ±1,2	—	100,0
II.	Pituitary stalk lesion	1—2 days	12	77,9 ±1,9	6,2 ±0,3	15,9 ±1,7	52,0 ±4,0	48,0 ±4,0
III.	Stalk lesion	8 days	12	73,2 ±1,9	14,3 ±1,0	12,5 ±1,2	34,6 ±2,1	65,4 ±2,1
IV.	Stalk lesion	More than a month	12	70,2 ±1,2	19,9 ±1,1	9,9 ±0,8	16,0 ±1,3	84,0 ±1,3

* Standard error

terations took place in the anterior pituitary. Due to the special blood supply of the pituitary CAMPBELL and HARRIS (1957) did not find necrosis in rabbits. In ferrets HOLMES (1961) noted only very slight necrosis. In monkeys the size of the necrotic area varied. (MAGOUN et al. 1939; HOLMES, 1962). A similar result was observed in humans (RUSSELL, 1956; LUNDBAEK et al. 1960). In the anterior pituitary of sheep and goats extensive necrosis could be demonstrated by DANIEL and PRICHARD (1957, 1958), as well as by ADAMS et al. (1963, 1964). Conserning rats, we only found few data in the literature. In agreement with BARRNETT and GREEP (1951) and DANIEL and PRICHARD (1956) we could also establish that if the volumetric examinations were performed 1—2 days after the operation almost the half of the anterior pituitary underwent necrosis.

Most authors merely studied this problem at a certain time following the surgical manipulation. Of course, this made it impossible to obtain an answer to the question of the fate of the alterations. Although, in the anterior pituitary there were indications of signs of fibrosis (MAGOUN et al. 1939; BARRNETT and GREEP, 1951; DANIEL and PRICHARD, 1958; HOLMES, 1961) the adequate investigation of the problem can only be solved by measurements accomplished at different periods after the operation. Our present investigations demonstrated that in the extensive decrease of the anterior pituitary volume the necrosis and consecutive fibrosis occurring after the stalk lesion played a decisive role, the volume of the surviving part of the gland also showed a slight decrease, an increase could not be observed in any case.

The data of the literature referring to the pars intermedia are very contradictory. Part of the authors reported an increase of the volume (UOTILA, 1939; BARRNETT and GREEP, 1951; DANIEL and PRICHARD, 1958; HÁMORI, 1960; HOLMES, 1962; ADAMS et al. 1964) others, on the other hand, did not observe a change (DANIEL and PRICHARD, 1956; CAMPBELL and HARRIS, 1957; HOLMES, 1961). According to HOLMES (1962) in monkeys the volumetric increase is a degenerative phenomenon. DANIEL and PRICHARD (1958) hold the view that in goats a real hyperplasia occurred. Our examinations showed that the increase in the volume of the intermediate lobe was only slight and biometrically insignificant; if the examination was carried out several weeks after the operation, due to the marked atrophy of the anterior and posterior lobe, relative hypertrophy could be observed in the pars intermedia.

Corresponding to the uniform findings of several authors (MAGOUN et al. 1939; BARRNETT and GREEP, 1951; DANIEL and PRICHARD, 1956, 1958; HOLMES, 1961, 1962; KOVÁCS et al. 1962; ADAMS et al. 1964), atrophy was observed in the posterior pituitary. Our investigations have also revealed that in rats the atrophy could only be confirmed some days after the lesion of the stalk. HOLMES (1961) made the same observation in ferrets and DANIEL and PICHARD (1956) in rats.

In accordance with the data of BARRNETT and GREEP (1951) the possibility arises whether the reduction of the volume of the surviving parenchyma — independently of hypothalamic relations — may play a role in the decrease of the trop-hormone production. It is known that following lesion of the pituitary stalk the hormone secretion of the anterior pituitary decreases (HARRIS, 1955; SZENTÁGOTHAI et al. 1962). Endocrine alterations

suggesting hypocorticism were also demonstrated by us, the results will be reported later.

To answer the above questions it should be stated that the decrease of the volume of the pars distalis cannot be the only causal factor in inducing adenohypophysial hypofunction. Our examinations demonstrated that almost half of the anterior lobe was destroyed owing to the lesion and the observations performed in partially hypophysectomized animals showed that the elimination of 50 per cent of the gland did not cause symptoms of endocrine deficiency (SMITH, 1932; GANONG and HUME, 1956; CAMPBELL, 1959). The studies of SHEEHAN (1937, 1948, 1961) and SHEEHAN and STANFIELD (1961) led to similar results. They analysed this question carefully in human material. They carried out the examinations on the pituitary glands of patients who died from postpartum pituitary necrosis and postpartum hypopituitarism and compared the morphological changes with the function of the pituitary gland.

Concerning the TSH function of the pituitary previous examinations showed that following lesion of the stalk a moderate hypothyroidism developed suggesting a decrease in TSH production (LÁSZLÓ et al. 1966). The weigh of the uterus and ovaries also decreased (DÁVID et al. 1966).

2. Hypophysial blood flow in rats after destruction of the pituitary stalk

It has been described above that after destruction of the hypophysial stalk in rats characteristic morphological changes took place in the pituitary gland. An extensive ischaemic necrosis was observed in the anterior lobe, later fibrosis and atrophy were seen in this area. The posterior lobe gradually atrophied. Considering that these alterations may be closely connected with the disturbances of the circulation of the gland it seemed worth while to study the blood flow of the pituitary in detail. For this purpose the India ink method as well as the ^{86}Rb method of SAPIRSTEIN and GOLDMAN (1958) were used.

The experiments were carried out on female albino rats of the same strain kept on a standard diet, weighing 180—220 g. The destruction of the pituitary stalk was performed in the manner described in the first chapter, under nembutal anaesthesia by means of a Horsley—Clarke apparatus. The location and extent of the lesion was checked by the procedure already described previously. Paraffin sections stained with haematoxylin and eosin were made from the corresponding portions of the hypothalamus. The animals in which the stalk was not, or only partly injured were discarded.

In some of the operated animals (16 rats), as well as in some of the controls (8 rats) under ether anaesthesia following exposure of the chest, India ink was injected directly into the left chamber of the heart 1 ml/rat (Pelikan, Günther, Wagner). Then, to stop the circulation a ligature was made at the base of the heart. Afterwards the rats were decapitated and autopsied. The brain being removed together with the pituitary was fixed in 4 per cent formalin and embedded in paraffin. 100 micron thick serial sections in the sagittal plane were made.

On combining the isotope method with the dye dilution procedure the following parameters were obtained: systolic blood pressure (mm Hg), weight of the anterior and posterior pituitary (mg); minute volume (ml/min/100 g body weight); the amount of blood flow of the anterior and posterior pituitary (microl/min/mg organ weight).

The procedures used by SAPIRSTEIN (1958) and GÖMÖRI et al. (1961), KÁLLAI and TAKÁCS (1961), GOLDMAN and SAPIRSTEIN (1958) and GOLDMAN (1963) were applied. The animals were fasted for 12 hr before the procedure, but tap water was freely available to them. Before the experiment the animals were anaesthetized with nembutal (4,5 mg/100 g body weight). Then both femoral veins and one of the carotid arteries were exposed and 0,1 ml heparin was injected iv. The systolic blood pressure was then measured with a mercury manometer by means of a polyethylene cannula inserted into the carotid artery. Following this a total amount of a 0,2 ml solution containing 10 mc ^{86}Rb as ^{86}RbCl was injected into one femoral vein and 20 sec later 0,2 ml of a solution of 1 per cent Geigy blue was injected into the opposite femoral vein. Immediately afterward blood samples from the carotid artery were withdrawn at intervals of 1 sec by means of a fraction collector. The blood had lost its blue colour after 20 sec and the rat was killed instantaneously by the iv administration of a saturated solution of potassium chloride. The skull was opened immediately and the brain and the pituitary were removed. The anterior and posterior lobes of the pituita-

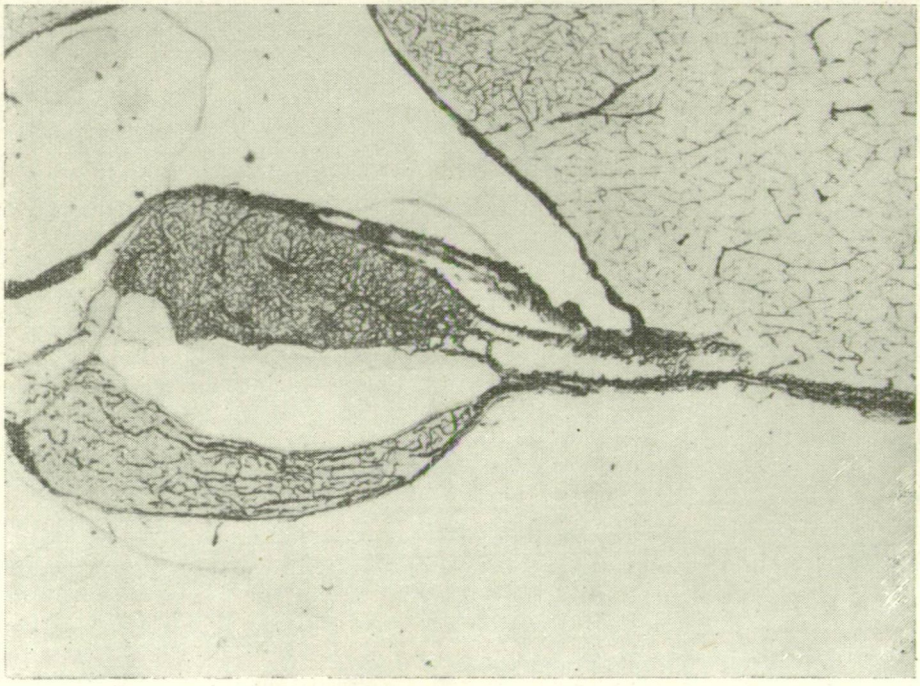


Fig. 7. Pituitary of intact rats after intracardial India ink injection. The hypothalamus is connected with the pars distalis by the portal vessels. 35 X.

ry were carefully separated and weighed and then hydrolysed in a 20 per cent solution of potassium hydroxide in a hot water bath. Their radioactivity was measured by a thallium-activated sodium iodine crystal. The values of the single organ fractions were expressed as a percentage of the total activity of the $^{86}RbCl$ solution given. The minute volume of the cardiac output was determined with the dye dilution method of HAMILTON et al. (1932). The Geigy blue concentration of the different arterial samples was measured by means of a Unicam spectrophotometer at 620 mμ wave length.

Student's „t" test was used in the statistical evaluation of the results.

First the microphotos demonstrating the results of the experiments performed with the India ink procedure are shown. In the intact rats the vascular connection between the hypothalamus and the pituitary, the portal vascular system, is well visible. The India ink uptake of both pituitary lobes is sharp and uniform (Fig. 7.). In the animals operated 1—2 days previously, this contact was interrupted and the India ink did not invade the central part of the anterior lobe and dye particles could only be found in some peripheral areas (Fig. 8.) On the other hand, in the rats in which the stalk had been destroyed some weeks earlier it may be seen that in addition to the extensive atrophy the vascular connection has been again restored as a result of the regeneration of the portal veins (Fig. 9.).

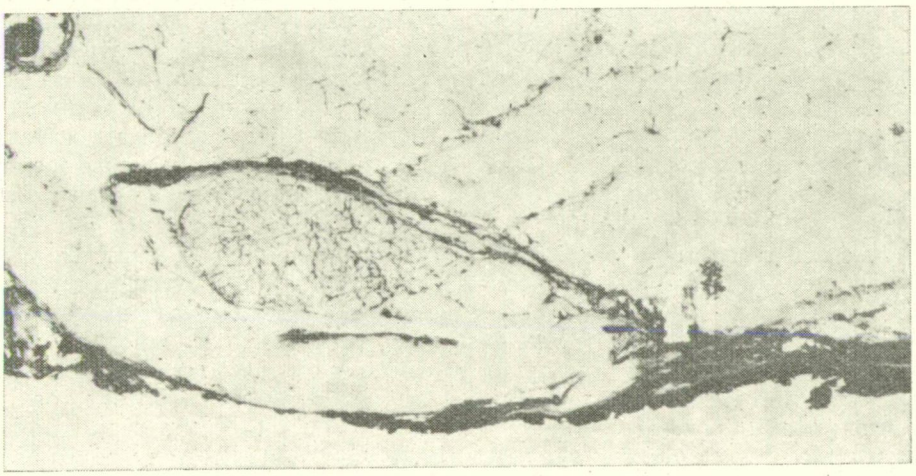


Fig. 8. Pituitary of rat with pituitary stalk lesion 1 day previously. The vascular connection between the hypothalamus and pars distalis is interrupted. After intracardial India ink injection. 35 X.

The result concerning the blood flow are presented on Table 3.

It could be established that in rats with a pituitary stalk lesion the blood pressure decreased, whereas the minute volume of cardiac output did not change appreciably. Two days after the operation the weight of the anterior and posterior lobes did not differ from that of the controls, but by four weeks the weight of both lobes had decreased significantly. In control rats the blood flow to the posterior lobe was about six to seven

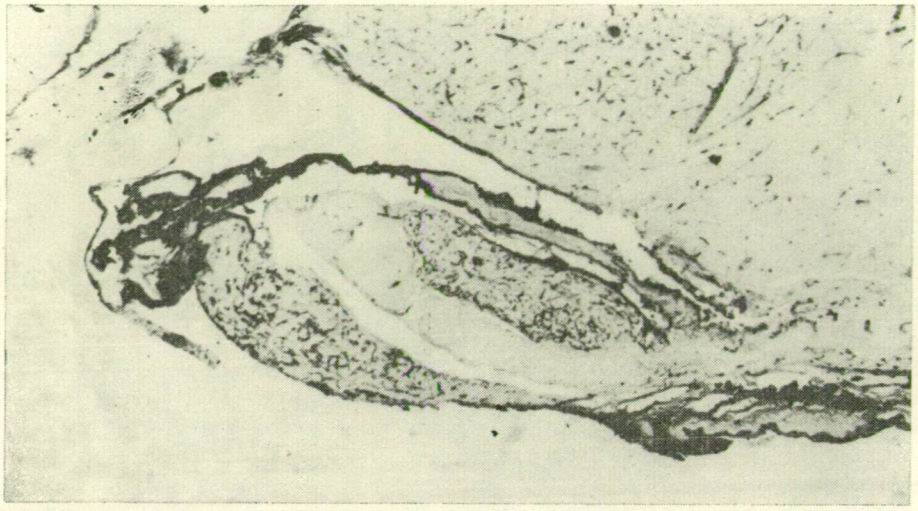


Fig. 9. Pituitary of rat with pituitary stalk lesion 1 month previously after intracardial India ink injection. The vascular connection between the hypothalamus and the pars distalis is restored. 35 X.

times as much as that to the anterior lobe. This finding agrees with that of GOLDMAN (1963). In rats with a lesion of the pituitary stalk the blood flow calculated according to the weight in mg of the anterior lobe was significantly reduced at two days. Four weeks after the operation the blood flow, on the other hand, did not change appreciably from that of the controls. In the gradually atrophying neural lobe the blood flow showed a decreasing tendency four weeks after the stalk lesion, however, this decrease was not significant as compared to the values of the controls because of a greater spread of the figures obtained.

As described above the study of the histological and volumetric changes showed that after destruction of the pituitary stalk ischaemic necrosis corresponding to infarcts developed in the pars distalis. On the average 52 per cent of the anterior lobe was destroyed. From the histological examinations it was evident that owing to the lesion not only the glandular cells, but also the vessels necrotised and the haemoglobin content of the erythrocytes disappeared. Hence, the necrotic part of the gland had no circulation. Accordingly, if the ^{86} Rb uptake was related to the weight of the anterior lobe expressed in mg, erroneous conclusions might have been drawn. It seemed better to express the ^{86} Rb uptake in terms of the surviving pituitary tissue. For this reason the volume of the infarct in each of the pituitaries used for the blood flow examinations should have been determined. However, this was technically not possible. Therefore, information concerning the size of the necrotic portion derived from another animal group was used. It is important to note that the volumetric examinations were performed on rats of the same strain subjected to the same type of stalk lesion. If therefore the blood flow was only related to the tissue of the surviving anterior lobe (48 per cent) it become apparent that the blood flow to the surviving anterior pituitary tissue was not decreased (Table 4.).

Table 3.

Blood pressure, minute volume and hypophysial blood flow of control rats and rats with pituitary stalk lesion

Control		Time elapsed after operation	No of animals	Body weight g	Blood pressure mm Hg	Weight of lobe mg: anterior	Weight of lobe mg: posterior	Minute volume ml/min/100 g body weight	Blood Flow micro l/min/mg tissve: Anterior lobe	Blood Flow micro l/min/mg tissve: Posterior lobe
I.	Control	—	12	209,3 ±4,2*	123,7 ±2,9	7,7 ±0,6	1,03 ±0,03	30,7 ±1,6	0,72 ±0,06	4,53 ±0,55
II.	Salk lesion	2 days	8	190,0 ±4,3	95,8 ±4,2	8,0 ±0,6	0,86 ±0,07	32,6 ±3,1	0,42 ±0,08	3,59 ±0,35
III.	Stalk lesion	4 weeks	15	192,0 ±3,8	90,9 ±4,2	4,7 ±0,5	0,37 ±0,04	28,2 ±2,2	0,66 ±0,09	4,40 ±0,56
Probability:				I/II.	$p<0,01$	$p>0,05$	$p<0,05$	$p>0,05$	$p<0,001$	$p>0,05$
				I/III.	$p<0,001$	$p<0,001$	$p<0,001$	$p>0,05$	$p<0,05$	$p>0,05$

* Standard error

Table 4.

Blood flow of the anterior pituitary in rats with pituitary stalk lesion referring to the surviving tissue of the anterior lobe

Group	No of animals	Blod flow of anterior lobe micro l/min/mg tissue
Control	12	0,72±0,06*
Stalk lesion *a)* calculated for the total anterior lobe tissue	8	0,42±0,08
b) Calculated for the surviving anterior lobe tissue	8	0,87±0,16

* Standard error

It was more difficult to evaluate the blood flow of the pars distalis of rats in which the pituitary stalk had been destroyed four weeks previously. By this time necrosis was no more visible, and the necrotic area was replaced by scarred connective tissue. The fibrotic portions corresponded to about 16 per cent of the anterior lobe. In this connective tissue intact vessels and erythrocytes were seen, and it was evident that the fibrotic area had some circulation. However, by means of histological studies the difference between the blood flow of this area and that of the intact gland amounting to 84 per cent in this period could not be established. At any rate a considerable decrease of the blood flow in the pars distalis of this experimental group could not be demonstrated by isotope examinations.

Our results show that two days and four weeks after the lesion of the pituitary stalk the blood flow of the tissue of the surviving anterior lobe and neural lobe was not reduced appreciably in rats. This result is, as a matter of fact, not surprising because if there were considerable decreases of the circulation hypoxia would have ensued and the surviving portion ought to have been also destroyed.

Before any conclusions can be drawn from the present results some possible errors of our method must be pointed out. It has been mentioned that the volumetric determinations and the blood flow examinations were not carried out in the same animals. This fact, however, cannot influence decisively the results as the operative techniques and the rat strain used were identical. It may also be erroneous that the circulation of the pars distalis of the animals operated four weeks previously was related to the whole anterior lobe and the fact was not taken into account that 16 per cent of the gland did not consist of intact parenchyma, but of scarred connective tissue. As, however, this part also possesses an adequate circulation it seemed preferable to consider this relatively not large portion as

the substance of the gland. The distribution of the ^{86} Rb uptake may also be influenced by the fact that the median eminence which was damaged in some of the cases traps about 20 per cent of the administered ^{86}Rb in intact animals (GOLDMAN, 1963).

Although, these sources of error must definitely be taken into consideration it seems certain that in the pituitary stalk lesioned rats the blood flow to the surviving tissue did not decrease to a great extent.

The most exciting question arising from these experiments concerns the route by which the blood reaches the peripheral surviving tissue of the anterior lobe. On the basis of the examinations performed this question cannot be definitely answered. Several possibilities must be taken into account. Perhaps the electrolytic lesion applied in the course of the examinations only damaged a part of the portal vasculature. As, however, the experiments performed by the India ink method uniformly showed that in the animals operated 1—2 days previously the hypothalamic-hypophysial vascular connections were totally interrupted, this assumption can be discarded. It should be noted that a similar extent of incomplete necrosis occurred when the stalk was cut by surgical means (DANIEL and PRICHARD, 1956; ADAMS et al. 1963); in this case the hypothalamic-hypophysial vascular connections were certainly totally interrupted. The second possibility was that regeneration of the portal vasculature took place and the direct link between the hypothalamus and the pituitary was restored. It is known that the portal vessels can regenerate rapidly (HARRIS, 1955). According to HARRIS (1950) the portal veins regenerate in rats already one day after surgical stalk section. Our experiments performed by the India ink method suggest that a few days after surgery a considerable regeneration of the portal vascular system does not ensue. This assumption is also supported by the histological pictures showing that in the necrotic tissue vascularisation did not occur. The restored portal circulation may play a definite role in the blood supply of the anterior lobe of the rats in which the stalk had been destroyed some weeks previously, however, it does not seem probable that immediately after the operation the surviving anterior lobe tissue gets its blood supply via the regenerated portal vessels. The difference between the results of HARRIS (1950) and ours might on the other hand, be due to the fact that following a simple stalk section the two ends of the pituitary stalk remain in close contact, whereas after electrolytic lesion the necrosis separates the two stumps of the stalk. This represents a certain extent of isolation and thus the conditions for the vascular regeneration were less good. A third possibility would be that the surviving peripheral portion of the anterior lobe obtains its blood supply from sources other than the portal system. This interpretation conflicts with the accepted view of the majority of the investigators (GOLDMAN and SAPIRSTEIN, 1962; GREEP, 1963). The data suggest that the anterior lobe of the rat lacks a systemic arterial blood supply and that its blood flow is completely provided by the portal vessels. According to our investigations the revision of this theory may be taken into consideration. Our experiments point to the fact that in addition to the portal circulation some other possibility must exist for the blood to reach the anterior pituitary. According to DANIEL and PRICHARD's (1957) observations in goats the main part of the pars distalis is provided by the

so-called long portal vessels contained in the stalk and as a result of their section the greater part of the gland becomes necrotic. In addition these authors presume the existence of another vascular system which they have termed „short portal vessels" being not damaged at the stalk lesion and may ensure the blood supply of the surviving tissue. DANIEL and PRICHARD (1956) explain the circulation of the surviving part of the anterior lobe in rats after lesion of the stalk with the following possibilities: 1. the blood may derive from the so-called peduncular arteries; the greater part of these vessels is in connection with the portal veins but they may also reach the pars distalis independently of the portal circulation. 2. The blood diffusing from the pituitary capsule into the parenchyma of the anterior lobe may also play a role. 3. Finally, the hypophysial posterior artery supplying directly the posterior pituitary may have branches to the anterior lobe. The latter possibility is also suggested by SZENTÁGOTHAI et al. (1957). They emphasized that it is not right to attribute the hypophysial blood supply exclusively to the portal circulation.

On the basis of the present investigations a definite conclusion cannot be drawn which of the enumerated possibilities may play an important role in the survival of the peripheral part of the anterior lobe. It may, however, be assumed that the blood supply of the pars distalis does not derive only from the long portal vessels.

Our investigations furnish further data regarding the problem of the regeneration of the adenohypophysis. The views of the investigators conflict on this subject. Some of the authors (SMITH, 1932; WEINBREN and FITSCHEN, 1959; HOLMES, 1961; KOVÁCS, 1961;; ADAMS et al. 1964) deny the possibility of a regenaration, others, on the other hand, (REISS et al. 1937; SAPIRSTEIN and GREEP, 1955; DANIEL and PRICHARD, 1958; ADAMS et al. 1963) observed signs pointing to regeneration. The experiments of DIERICKX (1964) should be mentioned who removed a portion of the anterior pituitary of frogs and observed that the surviving part being in connection with the portal vessels was completely regenerated by nine months. Our examinations showed that an enlargement of the volume of the surviving adenohypophysial tissue could not be observed in any case. Thus, the conclusion seems to be justified that the anterior pituitary remaining alive after the stalk lesion is not able to regenerate. Hence, concerning the blood flow of the anterior lobe the following questions arise: 1. Is the blood supply of the surviving part of the anterior lobe reduced. 2. Is the vascular connection restored between the pituitary and the hypothalamus which was interrupted following the lesion of the stalk. Relating to the first question it has been explained that the blood flow in the surviving anterior pituitary tissue did not decrease at all. The majority of the observations support the theory of the regeneration of the portal vessels (HARRIS, 1950, 1955; BARRNETT, 1951; ZUCKERMAN, 1955) this also holds for our own investigations. Therefore, it seems worth while to emphasise the observation that in spite of the adequate blood supply of the surviving adenohypophysial parenchyma and the reestablishment of the portal circulation, i. e. the restoration of the direct hypothalamo-adenohypophysial connections, the glandular cells of the anterior lobe are not capable of morphological regeneration. From the functional aspect this problem will be analysed in chapter VI.

Chapter III.

DISTURBANCE OF THE WATER METABOLISM FOLLOWING LESION OF THE PITUITARY STALK

1. Diuretic reaction developing after oral water loads, water intake, urinary output

The methods used for the examinations of the water metabolism were the determination of the diuretic reaction following oral administration of tap water and an isotonic solution of saline and the measuring of the daily spontaneous water consumption and urinary output.

The experiments were performed on male and female rats weighing 150—200 g kept on a standard diet. The surgical technique of the stalk lesion and the method of the histological control were described in the previous chapter in detail. For the evaluation of the results only those animals were used in which the stalk lesion was complete.

Before studying the diuretic reaction developing after the water load the rats were fasted for lo hours but tap water was alllowed ad libitum. At the start of the experiment tap water was withheld and the animals were placed separately in urine-collection cages, then tap water was given by stomach tube in quantities of 5 per cent/body weight. The other group of rats was given instead of tap water 0,9 per cent solution of NaCl (5 per cent/body weight). The urinary output was measured at hourly intervals for 8 hr with an accuracy of 0,1 ml. In earlier studies it was found (Kovács, G. S. et al. 1958; Kovács K. et al. 1959) that the intensity of diuresis could not only be plotted by means of the familiar diuresis curves (in a coordinate system, where the abscisse represents the time and the ordinate the amount of urine) but also by the use of a numerical value. This value has been called "summation urine output" and is essentially the sum of urine excretion related to the volume of water administered during 8 hours. The calculation is as follows:

The area under the summation polygon of the cumulative urine outputs measured at hourly intervals is multiplied with the reciprocal of the amount of water administered. Expressed as a formula:

$$S = \frac{1}{H} \left(\tfrac{1}{2} a_1 + a_2 + a_3 + \ldots\ldots a_7 + \tfrac{1}{2} a_8\right),$$

where S = summation urine output (the figure characteristic of the diuretic reaction) H = amounts of water administered per os, a_1, a_2, a_3 ..... a_7, a_8 = are the cumulative urine volumes measured in ml at the end of the first, second, third, ... seventh, eighth hours.

The second group of the animals was placed separately in urine collection cages and the water consumption during 24 hours and the spontaneous urinary output was measured with an accuracy of 0,1 ml.

The water metabolism of the rats was recorded continuously every day after the operation. This rendered possible to study the daily changes of the water metabolism of each animal. The results were biometrically analysed using Student's „t" test.

The results obtained concerning the changes of the summation urine output are presented on Table 5. The Table shows that there are consi-

Table 5.

Summation urine output following stalk lesion

Group	Times elapsed after operation in days	No of animals	Body weight g	Summation urine output	Probability
Control	—	30	179,8 ±5,3*	6,61 ±0,20	—
Stalk lesion	1	16	180,3 ±2,3	8,37 ±0,85	$0,02>p>0,01$
Stalk lesion	2	17	176,5 ±2,8	10,73 ±0,68	$p<0,001$
Stalk lesion	3	17	172,6 ±3,3	5,17 ±1,10	$p>0,05$
Stalk lesion	4	16	178,1 ±2,9	7,12 ±1,24	$p>0,05$
Stalk lesion	5	16	179,1 ±2,7	10,71 ±0,85	$p<0,001$
Stalk lesion	6	15	175,0 ±3,2	11,58 ±0,58	$p<0,001$
Stalk lesion	7	13	171,5 ±2,9	10,60 ±0,76	$p<0,001$
Stalk lesion	9	13	168,5 ±6,1	8,42 ±0,96	$0,02>p>0,01$
Stalk lesion	11	11	164,5 ±2,0	7,27 ±0,63	$0,02>p>0,01$
Stalk lesion	2 weeks	12	162,3 ±3,3	7,87 ±0,80	$p>0,05$
Stalk lesion	3—4 weeks	17	172,9 ±3,5	6,59 ±0,43	$p<0,05$
Stalk lesion	more than a month	25	190,4 ±4,1	6,52 ±0,35	$p>0,05$

* Standard error

derable alterations in the water metabolism of the stalk lesioned animals. Following the destruction of the stalk a transitory polyuria occurred, which, however, ceased on the 3—4. day and a diuretic reaction could not be elicited by oral water loads. On the fifth day the animals as compared to the non-operated controls again excrete significantly more urine. A more intensive diuretic reaction than the normal one lasts about 2 weeks then it gradually ceases and the average urinary output of the animals is essentially the same as that of the controls. As regards our data it should be noted that the average results contained in the Table are based on experiments performed in animal groups chosen at random. As significant individual differences in the water metabolism of the stalk lesioned rats took place mainly on the third and fourth day, the average values illustrated on the Table — at least of this period — are not quite reliable. This means that after acute polyuria a definite antidiuresis developed in each rat lasting generally one to two days. But this did not occur at the same time: in the majority of the animals it appeared on the third day, whereas a smaller part of the rats was still polyuric on this day. In some of the animals on the fourth day the oliguric phase had already ceased and the urinary excretion was again intensive; in some rats, on the other hand, antidiuresis could be observed within this period. Considering that the values shown in the Table indicate the average of the urinary output of the oliguric and polyuric rats, it is evident that the transient antidiuresis is in fact more marked. This was also demonstrated in Fig. 10. which represents

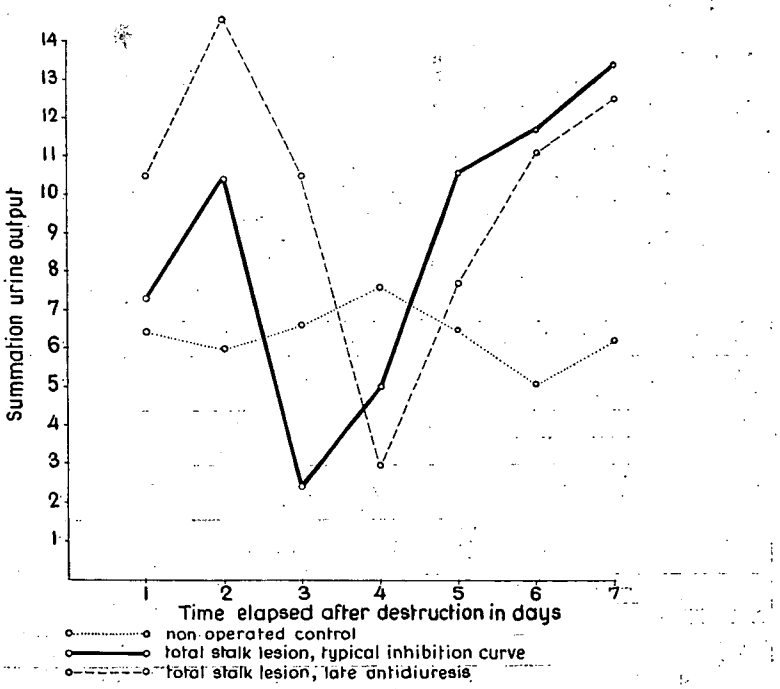
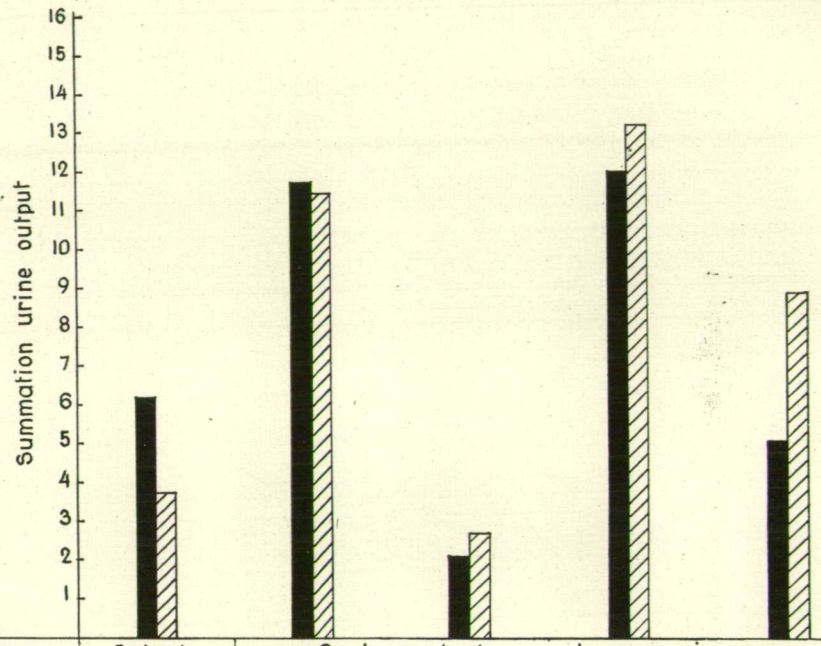


Fig. 10. Summation urine output of rats with stalk lesion following oral administration of tap water

the diuresis curve of a nonoperated control and that of two stalk lesioned rats on seven consecutive days. Whilst the diuretic reaction of the control animal did not show an appreciable difference on the different days, in the operated animals early polyuria developed. The appearance of the oliguric phase varies considerably; some of the animals still excreted urine in large amounts, whereas in the others the diuretic reaction was already not pronounced.

Returning to Table 5., it should be emphasized that in the enhanced diuretic period following the oliguric phase significant fluctuations occurred among the single animals. In part of the animals the diuretic reaction showed already a decreasing tendency in the second week, on the other



Group	Intact		Stalk lesion							
Fluid administered orally	Tap water	0·9 % NaCl	Tap water	0·9 % NaCl	Tap water	0·9 % NaCl	Tap water	0·9 % NaCl	Tap water	0·9 %
Time elapsed after operation (days)	—	—	2	2	3	3	5–7	5–7	More than 30 days	More than 30 days
Number of animals	20	20	18	14	12	10	28	22	22	21
Summation urine output	6·19 ± 0·18*	3·74 ± 0·38	11·76 ± 0·47	11·42 ± 0·69	2·10 ± 0·52	2·70 ± 0·57	12·05 ± 0·53	13·24 ± 0·60	5·10 ± 0·21	8·85 ± 0·62
Probability	—		$p \ll 0{\cdot}001$		$p \ll 0{\cdot}001$		$p \ll 0{\cdot}001$		$p < 0{\cdot}001$	
		—		$p \ll 0{\cdot}001$		$p > 0{\cdot}05$		$p \ll 0{\cdot}001$		$p \ll 0{\cdot}001$
	$p < 0{\cdot}001$		$p > 0{\cdot}05$		$p > 0{\cdot}05$		$p > 0{\cdot}05$		$p \ll 0{\cdot}001$	

* standard error

Fig. 11. Summation urine output of intact rats and in rats with stalk lesion (total stalk lesion) following oral administration of tap water and 0,9% NaCl.

hand, there were some rats in which still 3—4 weeks after the operation a polyuric reaction could be observed. These individual differences could not be correlated with the extent and localisation of the lesion considering that they also occurred in animals with a completely identical stalk lesion.

On Fig. 11. the results referring to the water metabolism of rats loaded with physiological saline or tap water can be seen. In accordance with our previous investigations (Kovács et al. 1959a) it could be established that in the non-operated controls only the administration of tap water brings about a diuretic reaction, in the case of physiological saline the excretion of urine is not so pronounced. In the stalk lesioned rats this difference between tap water or physiological saline loads could not be observed. In the polyuric phase the diuretic reaction was also very intensive following administration of physiological saline and it was remarkable that the oliguric phase was not prevented. Finally the observation noted on studying the water metabolism of rats operated some weeks previously should be mentioned. The investigations showed that whilst the diuretic reaction following an oral water load was already normal, by administration of physiological saline polyuria developed. Under such conditions the operated rats loaded with physiological saline excreted considerably more urine during the period of observation than the non-operated controls loaded with physiological saline or the rats with stalk lesion and loaded with tap water.

Fig. 12. shows the changes in the water intake and urinary output. The daily water intake of intact rats was on the average 25 ml and the sponta-

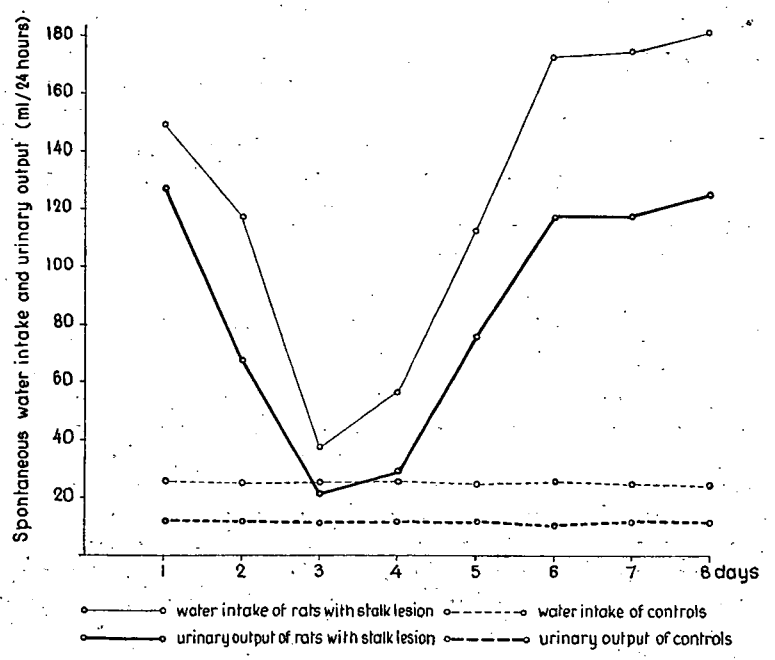


Fig. 12. Daily spontaneous water intake and urinary output of control rats and of rats with stalk lesion

neous urinary excretion about 11 ml. Considerable fluctuation could not be established on any of the experimental days. On the other hand, the water intake of the animals following lesion of the stalk markedly increased immediately after the operation, then it decreased again and on the third postoperative day the rats only drank approximately 35—40 ml tap water. Afterwards the polydipsia became gradually more pronounced. The curve of the urinary output ran nearly parallel to the water consumption.

The examinations suggest that following destruction of the pituitary stalk significant disturbances of the water metabolism ensued. The fluctuations among the animals were rather marked; nevertheless four phases could be distinguished: 1. a transient enhancement of the diuretic reaction and water intake, 2. an oliguric phase, 3. prolonged polydipsia and polyuria, 4. the diuretic reaction — at least in the case of oral water loads — apparently became normal.

The literature contains fairly few data referring to the changes in the water metabolism of rats following lesion of the hypophysial stalk. Numerous investigators assumed (McCann and Brobeck, 1954; Friedman et al. 1958; Alexander, 1958, 1959) that following destruction of the stalk intensive polyuria develops being evidently due to the lack of the antidiuretic hormone also in this species. It has, however, not been elucidated whether or not the diabetes insipidus ceases spontaneously or remains permanent. According to several authors (Pencharz et al. 1936; Alexander, 1958; Kennedy et al. 1963) after the damage of the stalk chronic diabetes insipidus develops, others (Swan and Penner, 1938) on the other hand suppose that prolonged polyuria cannot be elicited in rats. Our own experiments showed that the considerable polyuria observed during the water loads gradually ceased in some of the animals in the weeks after the lesion of the stalk. This apparent return to the normal state offered an interesting problem. Stutinsky (1951), Billenstien and Leveque (1955) and Moll (1957) found that in rats hypophysectomized some weeks previously the stump of the injured stalk regenerated and a new posterior pituitary-like structure developed. These morphological observations were supplemented by the investigations of Lloyd et al. (1954, 1955) who detected the appearance of the antidiuretic hormone in the blood under these conditions. Hence, it is possible that some weeks after the destruction of the stalk the production of the antidiuretic hormone starts again. In any case, the fact cannot be neglected that owing to the destruction of the stalk the anterior pituitary is also severely damaged, an extensive necrosis was seen in the pars distalis. The decrease of the function of the anterior lobe will be described below. The hypofunction of the anterior pituitary might also be the cause that the polyuric reaction fails to occur. This assumption is supported by the data of Von Hann, (1918); Richter, (1934); Kovács et al. (1959). These authors showed that the diabetes insipidus is abolished after the removal or destruction of the anterior lobe. However, these examinations could not definitely decide this question, further experiments were needed to analyse the problem. These investigations will be reported in the next chapter.

Chester-Jones (1957), Friedman et al. (1958) and Alexander (1959) found that the administration of NaCl induced an extensive polyuria in stalk lesioned or neurohypophysectomized rats. These observations were comple-

tely supported by our experiments, i. e. oral physiological saline loads also induced polyuria when the diuretic reaction following water administration reached already the normal level. In agreement with FRIEDMAN et al. (1958) we also found that NaCl administration is a simple method to confirm the efficiency or inefficiency of the stalk lesion. This simple procedure for the verification of the adequacy of the stalk lesion is more advantegeous than the water loads because in normal animals it only elicits a very mild diuretic reaction and thus on comparing the results with the data of the polyuric rats more pronounced differences could be observed.

Furthermore, it is worth while to mention the observation that the two methods used for studying the water metabolism — the diuretic reaction following oral water loads and the spontaneous water intake and urine output — did not change parallel in all cases. Hence, on the third day after the destruction of the stalk the diuretic reaction developing following the oral water load remained below the level of the control rats, whereas the water consumption and the urinary excretion was approximately the double of the values found in the non-operated animals. In a previous paper (LÁSZLÓ et al. 1963) this question was analysed in detail. It was established that the results obtained with the two procedures were influenced by different factors and that to demonstrate the liability to retain water the examination by means of water loads was more suitable. This assumption was supported by the observations obtained by the comparison of the diuretic reaction and water intake of rats in which the pituitary stalk was destroyed one month previously. It was also shown (KOVÁCS et al. 1959; DÁVID and KOVÁCS, 1962) that in hypophysectomized animals following oral water loads extensive antidiuresis occurred. Conversely, the spontaneous water intake measured in the same period was normal or even increased. Thus, the rats retained water after oral water loads, at the same time in accordance with the data of the literature (RICHTER, 1934; PENCHARZ et al. 1936; SCHWEIZER et al. 1943) the daily spontaneous water intake did not decrease.

According to the observations obtained on human material (HAGEL and KLAES, 1950; RANDALL et al. 1957, 1960; EHNI and ECKLES, 1959; SHARKEY et al. 1961) and the results of experiments performed on dogs and cats (FISHER et al. 1938; BELLOWS and WAGENEN, 1938; HEINBECKER and WHITE, 1941; HEINBECKER et al. 1947; PICKFORD and RITCHIE, 1945; O'CONNOR, 1952; CAMPAGNA et al. 1957; MUDD et al. 1957; BROOKS and PICKFORD, 1958) the disturbances in the water metabolism following the lesion of the hypophysial stalk occur in three phases. First an early polyuria develops. This is followed by a transient decrease of the urinary output, by the so-called normal interphase or antidiuretic phase. After this phase a permanent diabetes insipidus ensues. On rats the oliguric interphase was not investigated in detail. Only FRIEDMAN et al. (1958) drew attention to the possibility of an interphase. Earlier GERSH and BROOKS (1941) mentioned the occurrence of the phenomenon, however, neither of them carried out a systematic study in this field. Our own results proved beyond doubt that the oliguric phase (interphase) occurred also in rats following lesion of the pituitary stalk. During the oliguric phase diuretic reaction could not be elicited either by oral water or physiological saline loads. The mechanism of the interpha-

se was, however, not elucidated. It seemed, therefore, justified to perform further examinations to study this question.

2. The antidiuretic phase in rats following destruction of the pituitary stalk

In the experiments reported in the first part of this chapter it was shown that when oral water loads were applied and the water intake and urinary excretion of the rats were measured following destruction of the pituitary stalk a triphasic disturbance of the water metabolism ensued. First the water intake and the urinary volume increased and then transient oliguria lasting 1—2 days developed. The water retention was again followed by marked polyuria. We also referred to the fact that the tansitory water retention has already been described by other authors. The question, however, in rats has not been investigated thoroughly and it seemed advisable to study it in detail, since the cause of this characteristic and well reproducable phenomenon is not known. In this subchapter an account of the results of these experiments will be given.

The experiments were performed on albino rats of both sexes from the same strain weighing 170—210 g. The technique of operation used for the destruction of the stalk and the method for the histological evaluation have been described in the previous chapter. According to the morphological picture the rats were divided into groups with total and partial stalk lesions. In the former group only those rats were included in which both the proximal and distal parts of the stalk had been completely destroyed. The group with partial stalk lesion was composed of the animals in which one part of the proximal or distal portion of the stalk remained uneffected. In one group of the animals a unilateral focal hypothalamic lesion had been made in the praemamillary region, and in an other group a focal subcortical lesion was induced in the parietal area. In these animals the hypophysial stalk was not injured.

For the examination of the water metabolism the diuretic reaction developing after oral administration of tap water was determined, this method was described in detail at the beginning of the chapter. Some of the animals received instead of tap water physiological NaCl (5 per cent/body weight) or ethanol (5 per cent/body weight). Daily doses of 10 mg cortisone (Adreson, Organon, Oss) were applied for 8 days. This treatment started immediately after the operation. In this group 10 rats with total stalk destruction were used. For the sake of comparison the water metabolism of non-operated controls was also studied.

During the early polyuria, the antidiuretic phase and the polyuria following it, the specific gravity and the sodium, potassium, chloride and creatinine content in the urine of the rats with total stalk lesion was determined. The animals were given oral tap water or physiological NaCl loads, 5 per cent/body weight and the examinations were performed on the total amount of urine collected within 5 hours. The specific gravity of the urine was measured by means of a pyknometer with the method of GÁL et al. (1953), the sodium and potassium determinations were made with a flame photometer, that of chloride by the procedure

of SCHALES and SCHALES (1941), and the creatinine determination by the method of FOLIN-WU (1919) as modified by BROD-SIROTA (1948).

The volume of urine excreted in 5 hr. was computed for 100 g/body weight. The values of sodium, potassium and chloride are expressed in μ-equiv./l in addition the total amount excreted in 5 hr. expressed in μ-equiv. computed for 100 g body weight was also calculated. The creatinine values are expressed in mg creatinine output in 5 hr. computed for 100 g body weight. Finally the ratio of sodium and potassium (Na : K) was also estimated. The results were statistically analysed using Student's „t" test.

First of all it seemed necessary to examine whether the transient antidiuresis is or is not connected with the destruction of the pituitary stalk. In these experiments the water metabolism of rats with total or partial lesion of the stalk was compared with that of non-operated animals, and with animals with lesion which did not affect the stalk (subcortical or lateral hypothalamic lesion). The results are presented in Fig. 13. It is apparent that the oliguric phase occurs exlusively in the rats with stalk lesions; the diuretic reaction of non-operated controls and of rats in which the lesion did not affect the hypophysial stalk showed no major changes during the 7 days of observation. It is to be noted that the transient water retention occurred also in rats with partial lesion of the stalk, even when the lesion was so small in extent that no

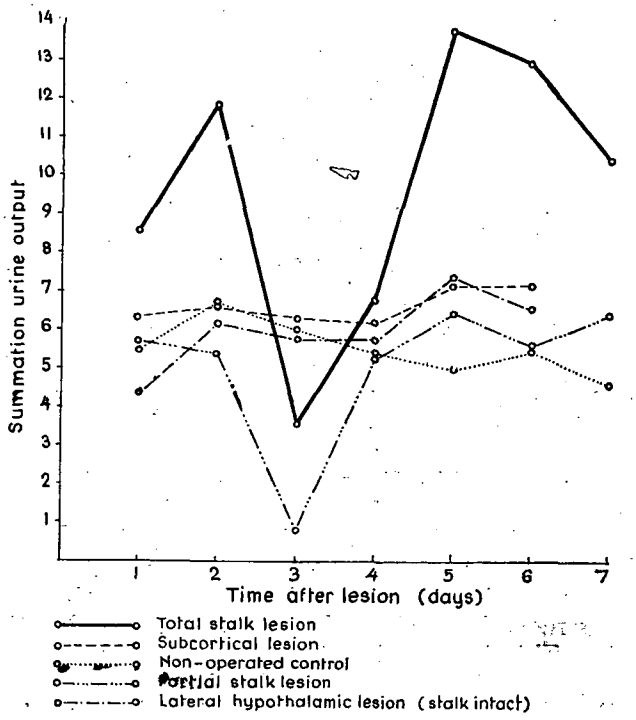


Fig. 13. Summation urine output in rats with stalk-, hypothalamic- and subcortical lesions following oral administration of tap water

polyuria developed. The water metabolism of rats with partially destroyed stalks is not discussed in this chapter; all that is mentioned here is that no oliguric phase was observed when lesions inflicted were very small.

Since the determination of the urinary volume and that of the intensity of the diuretic reaction give little information concerning the mechanism of the disturbance of the water metabolism it seemed worth while also to measure the changes in the specific gravity and the amount of Na, K, Cl, and creatinine output of the urine of rats with stalk lesion following oral tap water or physiological NaCl loads. The results are presented in Tables.

It may be seen in Table 6. that during the oliguric phase the osmolarity of the urine increased significantly, the specific gravity of the urine was higher than normal. The lowest values were observed in the polyuric phase, however, a marked difference as related to the controls could not be found. This is not surprising as the non-operated controls were also loaded per os with tap water. After the oral administration of physiological NaCl the specific gravity of the urine is somewhat higher both in the controls and in the rats in the oliguric phase. This increase cannot, or only hardly, be observed in the polyuric rats. The Table shows also the volume of the urine. The data indicate that the urinary output in 5 hr. computed for 100 g body weight significantly decreased also in these series.

In Table 7. the results concerning Na, K, Cl and creatinine excretion are presented. As the Table shows, during the oliguric phase the urinary Na, K and Cl concentrations increased in rats given either tap water or normal saline. However, as compared to the controls, urinary volume and electrolyte output decreased. Using water loading the Na : K ratio is somewhat higher than in the intact animals and the creatinine excretion is slightly lower. The last two changes could not be demonstrated when normal saline was administered per os. The polyuric rats showed considerable diuresis, a decrease in the concentrations of the electrolytes tested and an increase in the amounts of Na, K, Cl and creatinine excreted. The results from animals given saline solution differed from those in the rats given tap water, in that in every group of rats loaded with saline — least in the polyuric rats — the Na and Cl concentrations of the urine and the amounts of Na and Cl excreted were higher and the Na : K ratio increased. On the other hand, in intact rats given normal NaCl orally the urinary output was lower than in the controls hydrated with tap water. On days 1—2 and also on 6—8 after stalk destruction, the rats showed considerable diuresis even after administration of NaCl. The oliguric phase also occurred in rats given NaCl, though, at least in this series, the urine output decreased less than in the rats given tap water. Finally the results concerning the excretion of potassium also merit consideration. It is known that intact animals given normal saline excrete not only more Na and Cl, but also more K, than those administered tap water. This increased potassium excretion, however, does not occur in hypophysectomized animals, as has been demonstrated by Brunner et al (1956) and by Kovács et al. (1959). In the rats with stalk lesion the excretion of potassium after oral administration of a NaCl solution increased both in the polyuric and oliguric phases.

Table 7.

Sodium, potassium, chloride and creatinine outputs following oral administration of tap water and physiological NaCl solution in intact rats and in rats with stalk lesion

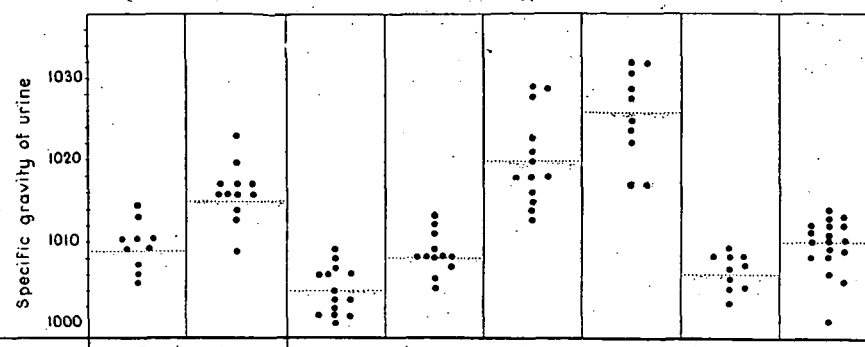
Group		Time elapsed after operation	Fluid administered	No. of animals	Body weight g	5 hr urine output calculated for 100 g body weight (ml)	concentration (m Equ/l.)			5 hour output calculated for 100 g body weight (μ Equ/100 g body weight)			Na/K ratio	5 hour creatinine output calculated for 100 g weight (mg)
							Na	K	Cl	Na	K	Cl		
I.	Intact	—	Tap water	10	168,0 ±3,3*	5,0±0,1	27,4 ±3,6	13,6 ±2,3	30,5 ±3,8	137,4 ±18,8	68,5 ±11,8	148,4 ±20,0	2,2 ±0,2	0,47 ±0,03
II.	Stalk lesion	1—2 days	Tap water	10	191,5 ±7,9	9,1±0,4	19,1 ±3,8	7,7 ±1,1	18,3 ±2,2	180,1 ±40,8	70,2 ±11,1	165,2 ±20,8	2,7 ±0,5	0,61 ±0,08
III.	Stalk lesion	3—5 days	Tap water	10	180,0 ±6,5	1,7+0,2	53,3 +7,9	21,9 ±4,5	60,3 ±11,0	83,6 ±13,1	34,9 ±8,4	100,2 ±22,2	3,2 ±0,7	0,38 ±0,30
IV.	Stalk lesion	6—8 days	Tap water	10	182,5 +5,1	9,2±0,7	22,6 ±2,4	8,7 ±1,0	19,9 ±2,1	208,9 ±29,7	76,2 ±6,4	178,5 ±19,4	2,9 ±0,5	0,63 ±0,03
V.	Intact	—	Phys. NaCl	10	178,0 +1,3	3,9±0,3	149,6 ±5,6	27,7 ±2,6	191,1 ±5,3	589,3 ±50,6	109,4 ±17,2	735,7 ±70,0	5,8 ±0,4	0,47 ±0,04
VI.	Stalk lesion	1—2 days	Phys. NaCl	10	197,0 +9,9	9,8±0,7	67,3 ±3,2	11,7 ±1,7	83,6 ±4,5	657,8 ±48,4	114,2 ±18,0	818,0 ±73,9	7,0 ±1,1	0,67 ±0,11
VII.	Stalk lesion	3—5 days	Phys. NaCl	10	192,5 ±5,7	2,5±0,3	161,9 ±1,0	33,1 ±4,1	204,9 ±13,5	399,3 ±42,1	79,6 ±12,3	493,7 ±62,0	5,9 ±1,0	0,54 ±0,04
VIII.	Stalk lesion	6—8 days	Phys. NaCl	10	205,0 ±8,6	10,2±0,7	68,3 ±5,9	13,6 ±1,6	86,0 ±6,4	656,3 ±40,8	131,9 ±13,8	841,5 ±45,9	5,5 ±0,6	0,70 ±0,15

* Standard error

Probability:									
I/II.	$p \ll 0{,}001$	$p > 0{,}05$	$0{,}05 > p > 0{,}02$	$0{,}02 > p > 0{,}01$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$
I/III.	$p \ll 0{,}001$	$p < 0{,}01$	$p > 0{,}05$	$0{,}02 > p > 0{,}01$	$0{,}05 > p > 0{,}02$	$0{,}05 > p > 0{,}02$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$
I/IV.	$p > 0{,}001$	$p > 0{,}01$	$p > 0{,}05$	$0{,}05 > p > 0{,}02$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$
V/VI.	$p \ll 0{,}001$	$p \ll 0{,}001$	$p < 0{,}001$	$p \ll 0{,}001$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$
V/VII.	$p < 0{,}01$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p < 0{,}01$	$p > 0{,}05$	$0{,}02 > p > 0{,}01$	$p > 0{,}05$	$p > 0{,}05$
V/VIII.	$p \ll 0{,}001$	$p \ll 0{,}001$	$p < 0{,}001$	$p \ll 0{,}001$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$
I/V.	$p < 0{,}01$	$p \ll 0{,}001$	$p < 0{,}001$	$p \ll 0{,}001$	$p \ll 0{,}001$	$p > 0{,}05$	$p \ll 0{,}001$	$0{,}02 > p > 0{,}01$	$p > 0{,}05$
II/VI.	$p > 0{,}05$	$p \ll 0{,}001$	$p > 0{,}05$	$p \ll 0{,}001$	$p < 0{,}001$	$p > 0{,}05$	$p \ll 0{,}001$	$p < 0{,}001$	$p > 0{,}05$
III/VII.	$0{,}05 > p > 0{,}02$	$p < 0{,}001$	$p < 0{,}05$	$p \ll 0{,}001$	$p < 0{,}001$	$p < 0{,}01$	$p < 0{,}001$	$p < 0{,}001$	$p > 0{,}05$
IV/VIII.	$p > 0{,}05$	$p < 0{,}001$	$0{,}02 > p > 0{,}01$	$p \ll 0{,}001$	$p \ll 0{,}001$	$p < 0{,}01$	$p \ll 0{,}001$	$p \ll 0{,}001$	$p > 0{,}05$

Table 6.

Specific gravity of the urine following oral administration of tap water and physiological NaCl solution in intact rats and in rats with stalk lesion



Group	Intact		with stalk lesion					
Orally administered fluid	Tap water	Phys. NaCl	Tap water	Phys. NaCl	Tap water	Phys. NaCl	Tap water	Phys. NaCl
Time elapsed after operation (days)	—	—	1 - 2	1 - 2	3 - 5	3 - 5	6 - 8	6 - 8
Number of animals	10	12	14	12	13	10	10	18
Body weight g	191·0 ± 2·8*	189·2 ± 4·6	191·1 ± 5·3	193·8 ± 4·6	178·8 ± 5·1	179·5 ± 2·4	173·5 ± 3·5	172·5 ± 1·6
5 hours urine output (ml) calculated for 100 g body weight	4·6 ± 0·1	2·7 ± 0·2	8·2 ± 0·2	8·3 ± 0·4	1·4 ± 0·1	1·3 ± 0·1	10·7 ± 0·6	10·0 ± 0·3
Specific gravity of urine	1009 ± 1	1015 ± 1	1004 ± 1	1008 ± 1	1020 ± 2	1026 ± 2	1006 ± 1	1010 ± 1

*Standard error

In a subsequent experiment an attempt was made to prevent the development of the oliguric phase. One group of rats with stalk lesions loaded orally with tap water (ten rats) were treated with cortisone; ten other rats were loaded orally with isotonic NaCl and ten rats with 5 per cent ethanol instead of tap water. These experiments, however, yielded negative results. As shown in Fig. 14. urinary excretion decreased after the early polyuric phase in each of the three groups. The data in the Fig. show that oliguria was apparently milder in the rats loaded with isotonic saline solution. The urinary output was similar to that of the intact and water loaded controls. It should be emphasized, however, that these mean values are not quite reliable. Antidiuresis developed in every rat with stalk lesion; since, however, some animals showed water retention on the third day and others on the fourth, the mean values calculated without selection are higher than the actual ones.

The present investigations prove the existence of a phase of transient antidiuresis following the early polyuria in rats with stalk lesion. This assumption is supported by the decrease of water consumption, by the absence of the diuretic reaction wich otherwise develops in response to oral administration of tap water and finally by the increase of the specific gravity, urinary sodium, potassium and chloride concentration. The only difference from other species is that while the interphase

begins in the cat and dog between the third and eleventh days and ceases 3—5 days later, in the rat it begins earlier (on the third or fourth day) and is shorter in duration (1 or 2 days).

The cause of the development of the antidiuretic phase is not clear. GERSH and BROOKS (1941) suggested that the injury of the hypothalamus-neurohypophysial system after destruction of the stalk is counter-balanced by the compensatory hypertrophy of the nerve cells producing

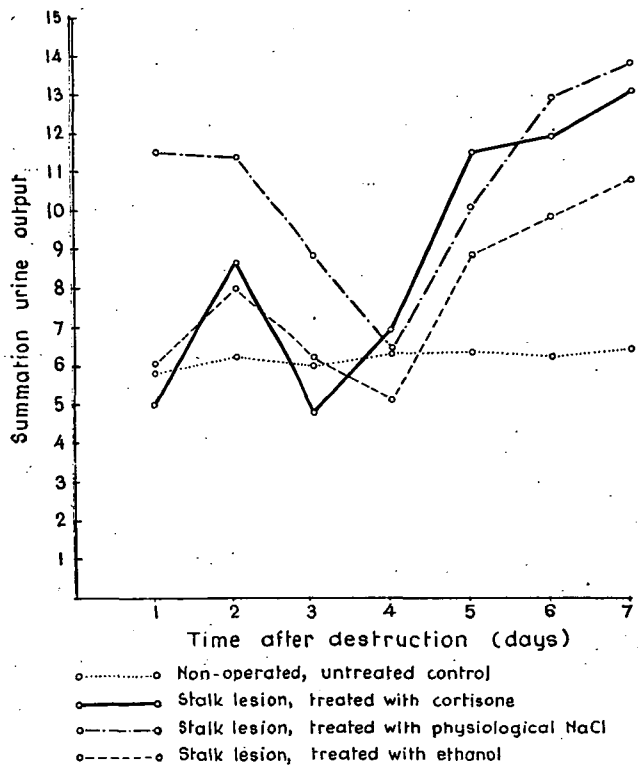


Fig. 14. Effect of cortisone, phys. NaCl or ethanol on summation urine output of rats with stalk lesions

ADH; it may also be assumed that following the operation well functioning nerve cells survived and later an „exhaustion atrophy" of the damaged neural elements ensued.

HEINBECKER and WHITE (1941) maintained that the antidiuretic hormone released from the neurohypophysis is responsible for the water retention. According to these authors the surgical trauma suspends for a while the secretion of ADH and thus polyuria results. The inhibitory effect of the trauma is short and the neurohypophysis soon becomes capable again of mobilizing ADH, which then results in water retention. The pituicytes soon degenerate as a consequence of the denervation resulting from the lesion of the stalk, ADH secretion ceases and a perma-

nent diabetes insipidus develops. O'CONNOR (1952) also attributes a role to ADH in the development of the oliguric interphase, but, unlike HEINBECKER and WHITE (1941) he believes that the mobilization of ADH is not a normal secretory process but a pathologic alteration due to the degeneration of the posterior lobe. MUDD et al. (1957) drew similar conclusions; according to these authors the cause of the antidiuretic period is an excessive and pathologic secretion of ADH and the phenomenon cannot be correlated with normal regulatory mechanisms.

The significance of ADH in the development of the oliguric phase is supported by a number of experimental observations. HEINBECKER and WHITE (1941) and KELLER (1942) showed that the interphase was absent in neurohypophysectomized or hypophysectomized dogs. In such animals polyuria was consistently observed immediately after operation. According to PICKFORD and RITCHIE (1945) and O'CONNOR (1952) in the dog during the interphase the urine became more concentrated, the specific gravity and chloride content increased, and no diuretic reaction could be induced by oral administration of water. It is known (FRIEDMAN et al. 1958; ALEXANDER, 1959) that in animals suffering from diabetes insipidus and possessing no supplies of ADH the oral administration of physiological NaCl solution evokes excessive polyuria. On the other hand, dogs in the interphase also excreted a smaller amount of concentrated urine when given saline orally. O'CONNOR (1952) has studied the antidiuretic activity of the urine in dogs with stalk lesions. His results seem to indicate that during the interphase an antidiuretic substance is present in the urine, while during the polyuric phase the urine does not contain ADH. Similar results have also been obtained by PHILLIPS and HARE (1945) who found that in cats with stalk lesions the ADH in the neurohypophysis decreased significantly only after permanent polyuria had developed.

The present investigations do not allow to draw any definite conclusions as to the cause of the development of the oliguric phase. At any rate, the results do not contradict the view that also in the rat, transient water retention may be caused by a mobilization of ADH. Moreover, the fact that the antidiuresis developed exclusively in animals with lesions of the stalk and was not observed in those with cerebral lesions of other locations also supports this view. It may also be stated that the decrease of the glomerular filtration rate or adrenal cortical hypofunction developing after destruction of the stalk (HARRIS, 1955/a) which may produce water retention (GARROD et al. 1955; DICKER, 1957; PETERS, 1959) cannot be the causative factors, because the rats with stalk lesions in the oliguric phase showed no major decreases in creatinine output and because the administration of cortisone did not suspend the interphase. The role of ADH is also not ruled out by the observation that the diuretic reaction could not be elicited following the administration of ethanol, which inhibits mobilization of ADH (EGGLETON, 1942, 1949). It is conceivable that ethanol can inhibit ADH secretion exlusively when the hypothalamo-pituitary system is intact. After lesion of the stalk the tissues producing ADH are obviously so severely damaged that the various impulses can no longer exert their effect.

3. *The role of the antidiuretic hormone in the antidiuretic phase of water metabolism in rats after lesion of the pituitary stalk*

The investigations described above showed that during the antidiuretic phase no diuretic reaction can be elicited by oral administration of tap water or NaCl, the urine becomes concentrated and its specific gravity, sodium, potassium and chloride concentrations increase, but creatinine excretion is virtually unchanged. It has also been demonstrated that water retention occurs exclusively when the hypophysial stalk has been damaged; focal cerebral lesions of other locations had no major influence on the diuretic reaction. On the basis of the results obtained it seemed justified to assume that in the induction of water retention the antidiuretic hormone released from the hypothalamo-neurohypophysial system damaged by the lesion of the stalk might play a role.

To confirm this assumption further experiments were found necessary. If ADH really plays a role in the genesis of the oliguric phase there will be no water retention if the antidiuretic activity of the hypothalamo-neurohypophysial system is diminished.

The experiments were performed on totally hypophysectomized and neurohypophysectomized rats and on rats which were neurohypophysectomized after destruction of the pituitary stalk. Then in rats previously deprived of water the stalk of the pituitary was destroyed; it is known that water deprivation leads to haemoconcentration and thus result in an almost total disappearance of the histologically demonstrable neurosecretory material from the hypothalamo-neurohypophysial system, as well as in a significant decrease of antidiuretic activity (SIMON, 1934; SIMON and KARDOS, 1934; ORTMANN, 1951; ZETLER, 1952; EICHNER, 1953; KOVÁCS et al. 1954; KOVÁCS, 1957). Finally the investigations were extended to a qualitative study of the neurosecretory substance contained in the hypothalamo-neurohypophysial system.

Albino rats of both sexes from the same strain weighing 180—210 g maintained on a mixed diet were used. The operative technique used for the stalk lesion was described previously. Total hypophysectomy and neurohypophysectomy were performed under ether anaesthesia by the pharyngeal approach, using the technique of SMITH (1932).

The groups of stalk lesioned, hypophysectomized and neurohypophysectomized rats were supplemented by three other groups of rats. In group 1. the neurohypophysis was removed on the day following destruction of the hypophysial stalk. In group 2. the stalk was destroyed after 8 days during which the rats were fed on a dry diet and drinking water was witheld. After operation the rats of this group were also allowed to drink tap water ad libitum. Group 3. contained intact control rats.

Except for the rats of group 2. all animals had been allowed drinking water ad libitum prior to operation and each received 10 ml of tap water by a gastric tube on each day of the week preceding operation. The purpose of the water administration was to ensure that the rats were not dehydrated.

Water metabolism was investigated by the method described at the beginning of this chapter. The degree of the diuretic reaction developing

after water loads was evaluated as summation urine output. The water balance studies began one day after operation and were continued daily for 7 days. The animals were then killed by decapitation. The success of the operation was ascertained by examining the area of the sella and by histological examination of the hypothalamo-hypophysial area. For this purpose the brain was removed together with the hypophysis fixed in Susa's solution, embedded in paraffin and serial sections 12 μ thick were mounted at 100 μ intervals. The sections cut in the frontal plain were stained with haematoxylin and eosin, and in several cases Gomori's chromalum-haematoxylin and aldehyde-fuchsin staining procedures were used. Thus, the results were evaluated from a total of 20 rats with complete destruction of the stalk, 10 hypophysectomized, 8 neurohypophysectomized, 6 with stalk lesions plus neurohypophysectomy, 6 water deprived with stalk lesions and 10 control rats.

The results of the experiments are presented in Figs. First the behaviour of the neurosecretory material was dealt with. The microphoto (Fig. 15.) shows the neurohypophysis of an animal one day after the stalk lesion stained by Gomori's chromalum-haematoxylin procedure; the considerable amount of neurosecretory substance located in the cells is well visible. When the oliguric phase ceased, in the present case in a rat killed on the fourth day after the operation — as can be seen on Fig. 16 —

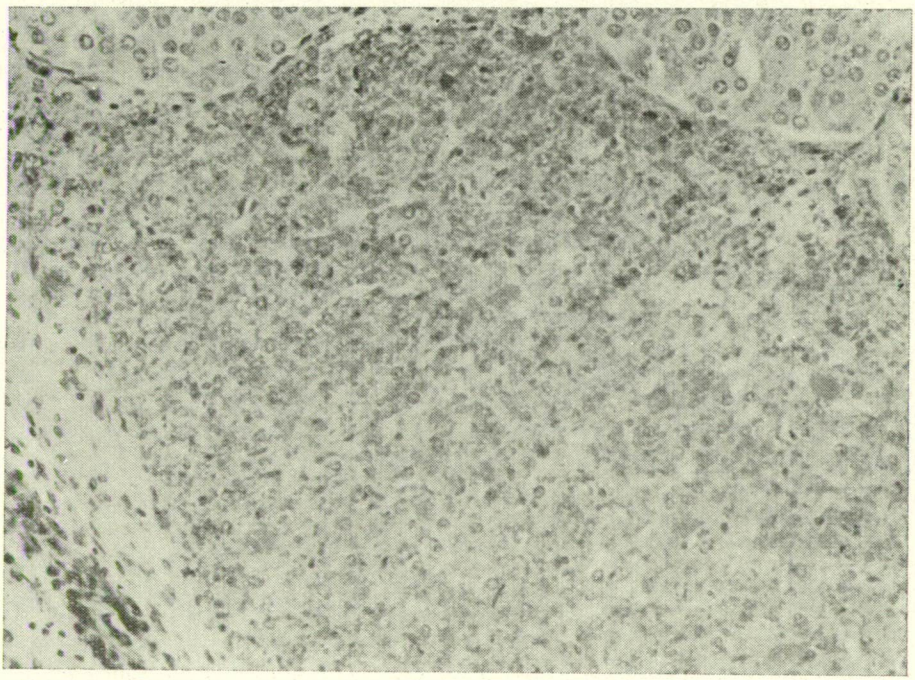


Fig. 15. Posterior pituitary of rat with stalk lesion performed a day previously. Many neurosecretory granules can be seen. Gomori's chrom-alum haematoxylin staining. 112 X.

the cytoplasm of the pituicytes is quite light, Gomori's positive substance has disappeared. Similar changes could be observed in the magnocellular nuclei of the hypothalamus; in the supraoptic nucleus, as well as in the magnocellular portion of the paraventricular nucleus.

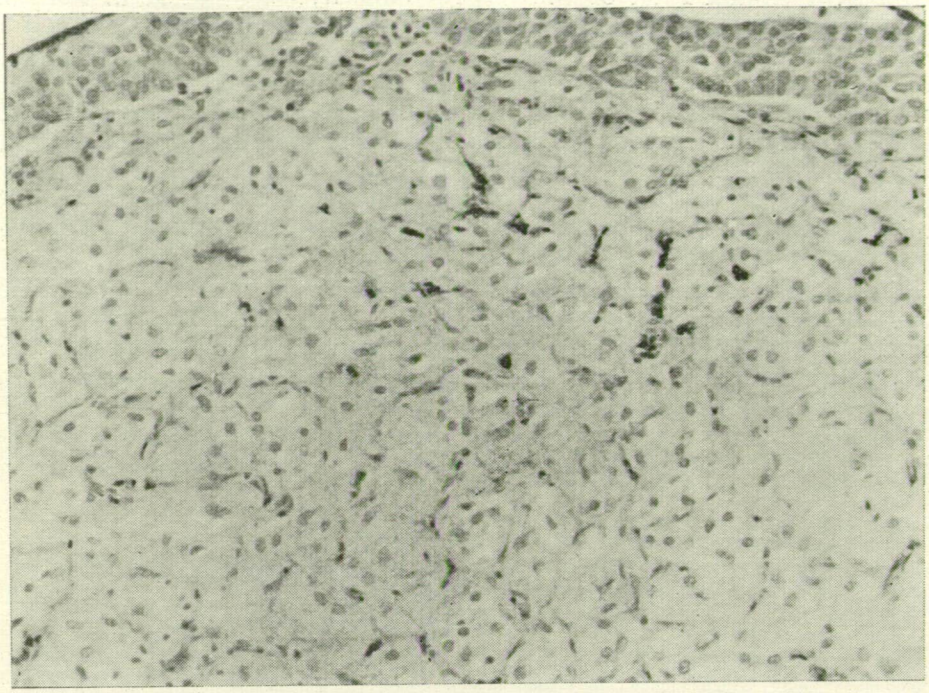


Fig. 16. Neurohypaphysis of a rat with stalk lesion 4 days after operation. The cells are quite ampty, the neurosecretory material has disappeared. Gomori's chrom-alum haematoxylin staining. 112 X.

From Fig. 17. it can be seen that the diuretic reaction (mean of the group) of the intact controls given tap water did not change significantly during the 7 days of the experiments. Rats with stalk lesions developed the triphasic disturbances of water balance described previously. At first, the animals excreted more urine than normal, then 3—4 days after operation water retention was observed, after which the diuretic reaction again became intense; polyuria could be observed. This triphasic disturbance of water metabolism did not occur in the hypophysectomized or neurohypophysectomized rats. In the former the short period of polyuria was followed by a gradual development of antidiuresis, the rats retained a significant proportion of the water administered (in the hypophysectomized animals the diuretic reaction did not return later to normal; consistent water retention ensued). In the neurohypophysectomized rats an excessive polyuria developed and this was not interrupted by antidiuresis. The oliguric phase was absent also when destruction of the stalk had been followed by neurohypophysectomy. Such animals excreted urine in much higher amounts than normal.

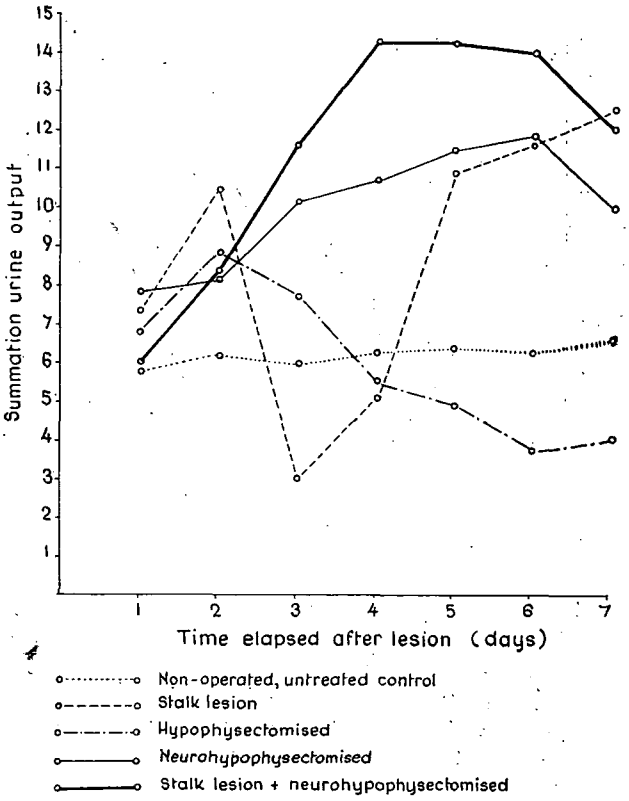


Fig. 17. Effect of hypophysectomy, neurohypophysectomy, neurohypophysectomy stalk lesion on the summation urine output of rats.

Fig. 18. presents the changes in the water metabolism of the rats deprived of water before destruction of the stalk. It can be seen that whereas in the rats allowed drinking water ad libitum and hydrated prior to the operation the initial polyuria was followed by a transient water retention, in the water-deprived rats the oliguric phase did not occur, and the volume of urine increased gradually.

The experiments indicate that all the procedures which diminished the antidiuretic activity of the hypothalamo-neurohypophysial system decrease or completely abolish the oliguric phase following lesions of the stalk. Thus, on the basis of the present and previous evidence it seems justified to conclude that in the development of the transient water retention which generally follows destruction of the pituitary stalk, ADH plays a very significant part.

It should be noted, that these investigations were performed in 1960—1961. The results were reported in 1962. The work of CRAWFORD and FROST (1963) on the same subject was published a year later. These authors confirmed our data and reached similar conclusions.

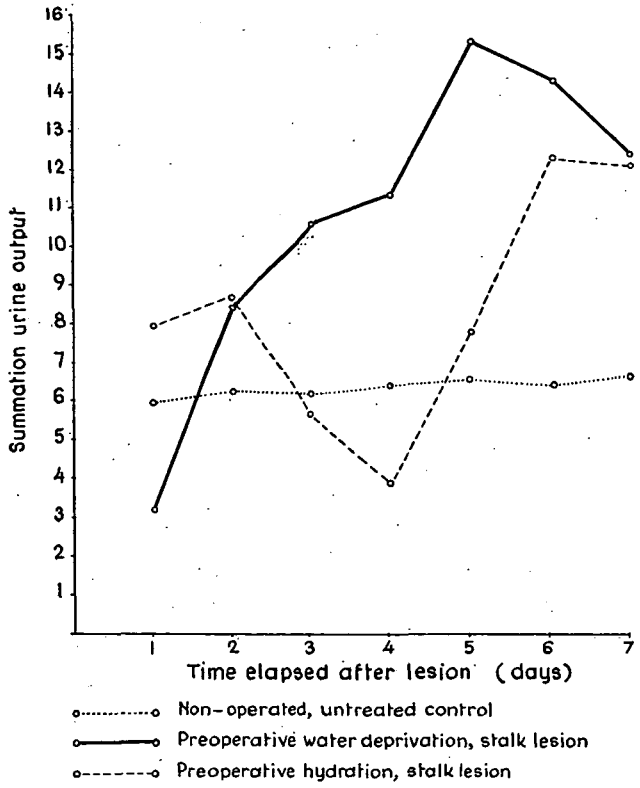


Fig. 18. Effect of pre-operative water deprivation on the summation urine output of rats with stalk lesion

The investigations have not clarified the mechanism by which ADH is released during the oliguric phase. The increased hormone mobilization may obviously be correlated with the processes of degeneration taking place as a result of stalk lesion in the hypothalamo-neurohypophysial system. We cannot explain, however, why the increased mobilization of ADH does not occur immediately after the stalk has been destroyed but only 3—4 days later, nor the factors that influence this process, which may be considered to be a pathological secretion.

4. ADH-excretion in the urine and the change of the ADH content of the hypothalamo-neurohypophysial system after lesion of the stalk. Functional capacity of the ADH system

On the basis of the experiments described in the previous subchapters it could be concluded that the development of the oliguric interphase is due to the effect of the antidiuretic hormone released from the hypothalamo-neurohypophysial system damaged following stalk lesion. The

change of the ADH content of the system has, however, not been subjected to exact quantitative examination after stalk lesion, therefore it seemed advisable to investigate the question in detail. In addition on the days following the operation the ADH activity of the urine of the rats was determined.

The experiments were carried out in 64, male albino rats weighing 180—210 g kept on standard diet. The stalk lesion was performed under ether anaesthesia by means of a modified Krieg apparatus (BOUMAN et al. 1957). At the end of the experiment the animals were decapitated and histologically evaluated as described previously.

The water metabolism of the animals was estimated by the measurement of the spontaneous water intake and urinary excretion during 24 hours.

Hyperosmosis was induced by the subcutaneous administration of 1,2 ml of a 15 per cent NaCl solution containing 2,4 per cent procaine HCl to avoid pain (WAYNER et al. 1964). After the treatment the drinking water was withheld and the urine was collected during 6 hours. The 6 hours period preceding the injection served as control period. The extent of the urinary secretion of ADH was determined by the samples obtained in this manner.

The antidiuretic hormone was biologically titrated according to the method of DE WIED (1960). Albino female rats weighing 140—160 g were used for the examination. Before the experiment was started the animals were hydrated with 0,2 per cent NaCl three times weekly for three weeks per os by a gastric tube that they should get used to the manipulation. On the day of the examination a solution containing 2 per cent ethanol and 0,2 per cent NaCl was administered (5 per cent/body weight) also by a gastric tube, one hour later the animals were anaesthetised with 10 per cent ethanol in the same manner using the same amount, and two hours after the beginning of the experiment the rats were again hydrated with a diluted ethanol solution. After this pretreatment a polyethylene cannule was inserted into the bladder and the excreted urine was measured in intervals of ten minutes. A 2 per cent ethanol solution corresponding to the amount of excreted urine was added per os. The preparations were administered into the tail vein and the decrease of diuresis was expressed in per cent. A 3-point assay was carried out whereby the ADH potency of the unknown was determined between 10 and 30 micro U of the standard (lysine vasopressin 300 I. U./mg, N. V. Organon, Oss).

ADH content of the whole hypothalamus or posterior lobe was determined after extraction with 0,1 N HCl. The substances were grinded with 0,2 ml 0,1 N HCl. The supernatant was neutralised with 0,1 N NaOH and the homogenate was centrifuged at 3000 RPM for 10 minutes.

Urine samples were also adjusted to pH 7 by treatment with 0,1 N HCl made isotonic by addition of various quantities of sodium chloride. Not more than 1 ml of urine was injected into the assay rats. The glass jars used for the sampling of urine contained 1 ml of 0,1 N HCl to keep the urine acid.

The results were biometrically analysed by Student's „t" test.

First the ADH activity of the urine was determined on several days following the lesion of the stalk. The results are presented in Fig. 19. It may be seen in the Fig. that on the first and second days the ADH excretion of the operated animals was less than that of the non-operated controls. On the third day following surgery there was a moderate, on the fourth day a definite rise, then the urinary ADH activity decreased again and on the sixth day it reached its minimum.

A comparison of the values of the spontaneous water intake and the urinary output, as well as those measured after water loads with the ratio of the ADH excretion in the urine showed that the results are generally in agreement. Only between the parameters found on the third and fourth days following lesion of the stalk a certain contradiction could be observed. Whereas the water consumption, urinary excretion and diuretic reaction after water load were the lowest on the third day the ADH excretion was the highest on the fourth day of the experiment. The cause of this phenomenon cannot be explained by these investigations. It is possible that the fact — already mentioned at the beginning of this chapter — that individual divergences occurred in the water balance of the animals on the third and fourth day following lesion of the stalk played a

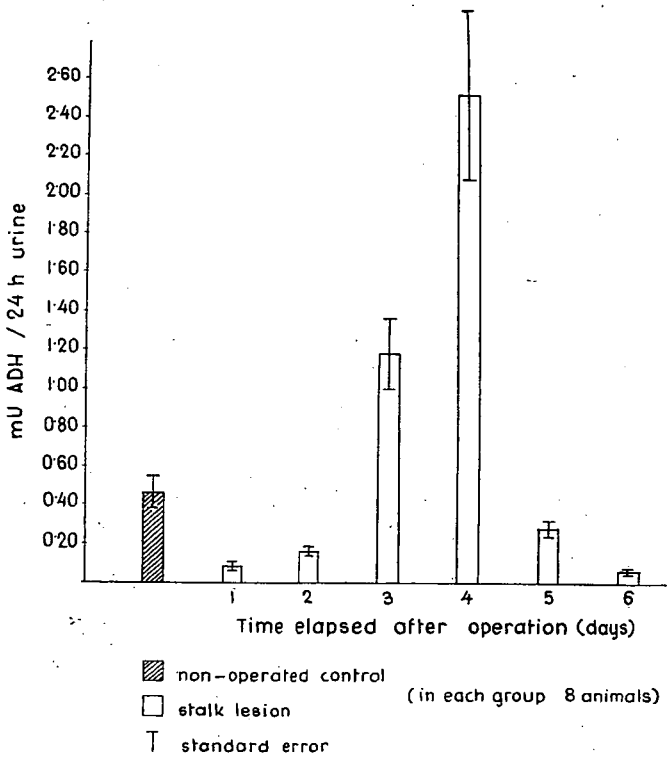


Fig. 19. ADH secretion in non-operated control rats and in rats with pituitary stalk lesion

role, therefore, the mean values illustrated in the figures — at least of this period — are not quite real. The lowest value of the oliguric interphase did not always occur on the third day but often ensued later. In these rats the maximum of the hormone excretion also shifted and these data influenced to a great extent the mean values. However, the question cannot be considered to be elucidated; further experiments are needed to decide the problem adequately. The results are reported in the following subchapter.

Subsequently it seemed advisable to draw a conclusion concerning the amount of the antidiuretic hormone released from the hypothalamo-neurohypophysial system after the lesion of the stalk. Hence, the ADH content of the neurohypophysis and the hypothalamus was examined in the oliguric interphase as we assumed that these values would give information concerning the quantity of the excreted hormone. The results are summarised on Table 8. It may be seen that the weight of the posterior lobe decreased gradually after the operation, the extent of the atrophy is the most marked on the seventh day and the loss of weight is more than 60 per cent of the control value. On the first day following the lesion the ADH content of the posterior pituitary did not show a significant change. The most pronounced decline occurred in the interphase and subsequently the divergence decreased. According to our investigations the neural lobe lost approximately 1 IU of ADH during seven days. The hypothalamus contained approximately 10 times less antidiuretic hormone and its content changed parallel with the ADH activity found in the neurohypophysis after destruction of the stalk.

Finally, the functional capacity of the adiuretin system was studied by examining the ADH excretion of nonoperated controls and that of stalk lesioned animals after treatment with hypertonic NaCl. The data are demonstrated in Table 9. It may be seen on the Table that following hyperosmosis the diuresis of the controls and the rats submitted to surgery showed discrepancies, whereas in the non-operated control group, in spite of the deprivation of water, the urine excretion increased, in polyuric stalk lesioned animals the amount of the urine decreased to the half. During the period of dehydration the ADH excretion of the intact animals was considerably higher than in the preceding control period. On the other hand, in the rats in which the stalk had been destroyed — when the experiment had been performed on the first or seventh day after the operation — the total amount of urinary ADH excretion only increased moderately. The examinations suggest that if the pituitary stalk was damaged the ability of the rats to mobilise ADH was inhibited both before and after the interphase.

After reporting the results the question arises if the method of the ADH determination used by us can be considered as specific, i. e. whether the antidiuretic activity of the urine, the posterior lobe and the hypothalamus depends on its ADH content. To decide this question the hypothalamus and the extracts of the posterior lobe were treated at room temperature for 30 minutes with 0,01 M thioglycollate and the urine with 0,08 M of this compound. By means of this procedure we succeeded in inactivating 96 per cent of the urinary ADH activity, 90 per cent of the anti-

Table 8.

Weight of neurohypophysis, ADH content of the hypothalamo-neurohypophysial system in intact rats and in rats with pituitary stalk lesion

	Group	Time elapsed after operation (days)	No. of animals	Body weight g	Weight of neuronypophysis mg	ADH content of neuro-hypophysis mU	ADH content of hypothalamus mU
I.	Intact	—	8	203,7±6,8*	0,90±0,05	1120±55	111±15,0
II.	Stalk lesion	1	8	199,5±7,2	0,89±0,04	1050±45	98± 7,5
III.	Stalk lesion	3	8	209,3±7,7	0,61±0,03	480±25	57±11,0
IV.	Stalk lesion	5	8	204,0±6,9	0,46±0,05	135±15	18±2,5
V.	Stalk lesion	7	8	196,4±6,0	0,35±0,03	120±12	16±2,5
				I/II.	$p>0,05$	$p>0,05$	$p>0,05$
				I/III.	$p\ll0,001$	$p\ll0,001$	$p<0,01$
			Probability:	I/IV.	$p\ll0,001$	$p\ll0,001$	$p<0,001$
				I/V.	$p\ll0,001$	$p\ll0,001$	$p<0,001$

*Standard error

Table 9.

Effect of hyperosmosis on the urinary output and ADH secretion of non-operated control rats and rtats with pituitary stalk lesion

Group		Time elapsed after operation	No. of animals	Amount of urine ml/6h		ADH-secretion mU/6 h		Probability *a/b*
				before dehydration	after dehydration	*a)* before dehydration	*(b* after dehydration	
I.	Non-operated	—	8	2,4 ±0,441*	6,5 ±0,236	0,14 ±0,013	1,99 ±0.375	$p \ll 0{,}001$
II.	Stalk lesion	1 day	8	22,1 ±2,300	11,2 ±0,560	0,06 ±0,021	0,16 ±0,024	$p < 0{,}01$
III.	Stalk lesion	7 days	8	29,9 ±9,951	13,1 ±0,585	0,019 ±0,04	0,10 ±0,029	$p > 0{,}05$
*Standard error			Probability: I/II.	$p \ll 0{,}001$	$p < 0{,}001$	$p < 0{,}001$	$p \ll 0{,}001$	—
			Probability: I/III.	$p \ll 0{,}001$	$p < 0{,}001$	$p < 0{,}001$	$p \ll 0{,}001$	—

diuretic activity of the hypothalamic extract and 87 per cent of the hormone activity of the neural lobe. The results of these supplementary experiments suggest that the antidiuretic hormone contained in the samples is responsible for the ADH effect measured during the procedure.

The results of the present experimental series (following stalk lesion the urinary ADH excretion increased, the adiuretin content of the neurohypophysis and hypothalamus decreased) furnished further evidence that the antidiuretic hormone released from the degenerating hypothalamo-neurohypophysial system played an important role in the development of the transient oliguric period following impairment of the stalk. Furthermore, conclusions may be drawn about the quantity of hormone responsible for the elicitation of the phenomenon.

The observation that immediately after the lesion the ADH content of the posterior pituitary and hypothalamus remained unchanged and that the considerable reduction of the hormone could only be demonstrated after the oliguric interphase, seemed remarkable. The investigations of PHILLIPS and HARE (1945) showed similar results. It is interesting that in the first phase of the disturbance of the water metabolism the daily water intake and diuretic reaction of the animals rose significantly. Presumably the cause of this change was that following the usual physiological stimulation the antidiuretic hormone contained in the ADH centre could not reach the circulation in adequate amounts — obviously owing to the interruption of the continuity of the supraoptic-hypophysial tract. This assumption was supported by the experiments showing that after treatment with hypertonic NaCl, the ADH excretion did not increase appreciably during the period of the transient polyuria. Unlike HEINBECKER and WHITE (1941) who explained the interphase with normal ADH excretion, our examinations suggest that in agreement with the hypothesis of O'CONNOR (1952) and MUDD et al. (1957) transitory water retention developed as a consequence of excessive and pathological ADH secretion from the adiuretin centre damaged owing to the lesion of the hypophysial stalk and that the hormone release is not in correlation with the normal regulatory mechanisms.

5. *The effect exerted by the preparations containing various antidiuretic hormones on the water metabolism of polyuric rats. Posterior pituitary-transplantation*

The release of antidiuretic hormone from the hypothalamo-neurohypophysial system in rats with stalk lesions corresponded according to the examinations reported in the preceding chapters to the changes observed in the spontaneous water intake and urinary output. A comparison of the changes of these parameters and the urinary ADH excretion showed, however, certain discrepancies. On investigating the problems further it seemed worth while to study how the different kinds and various amounts of vasopressin preparations influence the water metabolism of polyuric rats not having supplies of adiuretin. Therefore, substances containing ADH were administered to animals suffering from

diabetes insipidus and after the treatment the daily water consumption and ADH excretion were measured parallel. The experiments were extended to the examination of the effect of neurohypophysial transplantation into polyuric stalk lesioned rats.

For the experiments in which lesions had been made 46 male albino rats weighing 200—250 g were used. The stalk lesion was performed under ether anaesthesia by means of the modified Krieg apparatus BOUMAN et al. 1957). At the end of the experiments the animals were decapitated and histologically evaluated as described previously.

The water balance of the rats was estimated by measuring the spontaneous water intake and urinary output collected in 24 hours. The rats submitted to surgery 2—6 weeks previously were placed in urine-collection cages and the above parameters were recorded continously. As, however, according to our experience the curve of the urinary excretion ran always parallel with that of the water intake, for the sake of simplicity it was not illustrated on the Figs.

The ADH determinations were performed in female rats under ethanol anaesthesia in the way described in the previous part, where it has also been reported how the urine samples were prepared for the measurement of the adiuretin content.

The vasopressin preparations were administered subcutaneously. In some experiments only partially hydrolyzed gelatine was used as control. To prepare a long acting preparation one part of the peptide dissolved in distilled water was added to 3 parts of gelatine.

The following preparations were used: purified lysine vasopressin (300 I. U./mg) (Organon, Oss), purified arginine vasopressin (400 I. U./mg) (Organon, Oss), Pitressin tannate in oil (Parke, Davis, Hounslow).

In another experiment the effect of implantation of the neurohypophysis under the kidney capsule was studied. For this reason posterior lobes from intact adult rats were removed from the sella after decapitation of the animals and placed into cold isotonic saline solution. The left kidney of rats with stalk lesions was exposed under ether anaesthesia through a lateral skin incision and a small hole was made under the kidney capsule. In some cases the animals were operated by a similar procedure, however, without actually placing the neurohypophysis under the kidney capsule; these animals were used as controls; at the end of the experiment the rats were decapitated and the implants were inspected, dissected from the kidney and embedded in paraffin. Sections (6μ) were stained with aldehyde-fuchsin for microscopical analysis.

The results are presented in several Figs. First the effect exerted by lysin vasopressin on the water metabolism was studied. The polypeptide was given in different forms and doses to the animals. One group only received 1,0 I. U. of the purified preparation, in other cases, on the other hand, to make the effect of the preparation long lasting the vasopressin was mixed with gelatine. The doses used were 0,1, 1,0 and 4 x 0,25 I. U., in the latter case the rats were treated for two days at intervals of 12 hours. Fig. 20. shows, how this ADH preparation influenced the spontaneous water consumption of the polyuric animals during 24 hours. As can be seen on the Fig. the short acting purified preparation did not induce

any change in the curve of the water intake even in a dose of 1,0 I. U. If the same dose (1,0 I. U.) was mixed with gelatine the effect was more pronounced, however, the water intake only decreased for 8—10 hours, on the second day after the treatment the polydipsia was again more marked. Similar changes could be observed after the administration of 0;1 I. U., however, the decrease of the water consumption was more moderate. The effect of the polypeptide was far more marked if a permanent vasopressin level was maintained by injections given at intervals of 12 hours.

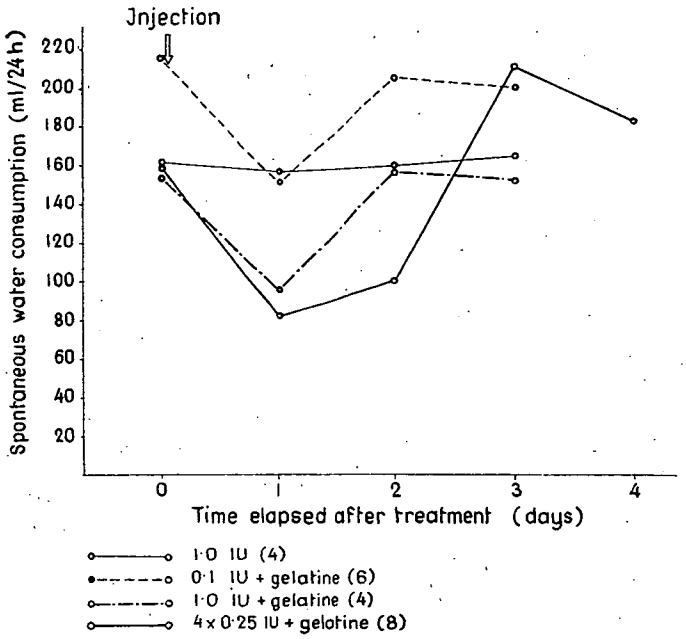


Fig. 20. Effect of lysine vasopressin on water intake of rats with stalk lesion

Thus, the urinary ADH content also only showed a rise during one day. The group to which the preparation was administered four times was an exception. Fig. 21. demonstrates that the recovery of the hormone from the urine did not change linearly with the dose applied. A comparison of the height of the columns showed that after mixing the compound with gelatine the ADH activity of the urine was the double of the activity observed following treatment with the same amount (1,0 I. U.) without gelatine. If a smaller dose was injected relatively more adiuretin was excreted. It is interesting that the water consumption of rats treated for two days already showed an increasing tendency on the second day of the therapy, at the same time more ADH was found in the urine. A certain similarity could be discovered between this phenomenon and the alterations observed when the spontaneous hormone excretion in the oliguric interphase and the water consumption were computed.

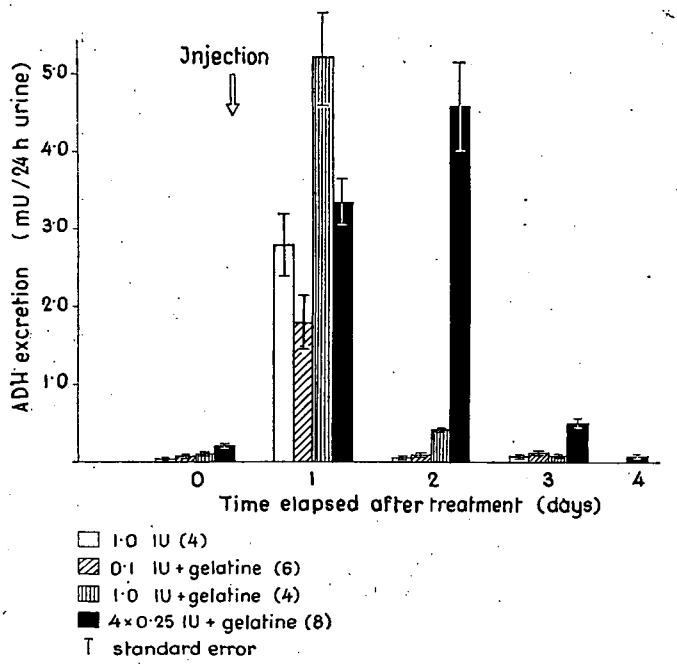


Fig. 21. Effect of lysine vasopressin on ADH excretion of rats with pituitary stalk lesion

A study of the effect of arginine vasopressin revealed that the change of the two parameters examined corresponded to the effect of the same dose (1,0 I. U.) of lysine vasopressin (Fig. 22.).

When we compared the divergences caused by doses of 1,0 and 0,5 I. U. Pitressin tannate (Fig. 23.), it could be noted that in both cases following the treatment the animals consumed far less tap water, however, after administration of 1,0 I. U. the water consumption only reached after some time the original level. As to the urinary ADH excretion after both doses the adiuretin content increased considerably. On the second or third days following the injection, however, a remarkable difference could be observed in the two groups: whereas the urinary ADH activity of the rats treated with 1,0 I. U. rose visibly, after administration of 0,5 I. U. this change was only very slight within the same period.

Fig. 24. demonstrates the results of neurohypophysis transplantations. The data revealed that the influence of the graft exerted on the water metabolism of rats with stalk lesion could be observed for about three days. The curve of the water intake reached its minimum on the first postoperative day and subsequently it showed a gradually increasing tendency. On the first and second days following implantation of the posterior pituitary under the kidney capsule the amount of excreted ADH showed almost the same rise, on the third day the values declined and within the following 24 hours the adiuretin content of the urine fell below

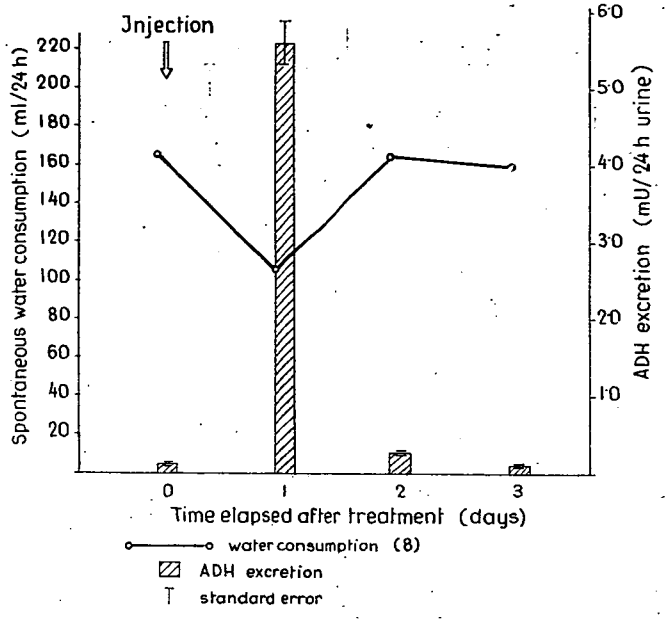


Fig 22. Effect of arginine vasopressin (1 IU) on water consumption and ADH excretion of rats with pituitary stalk lesion

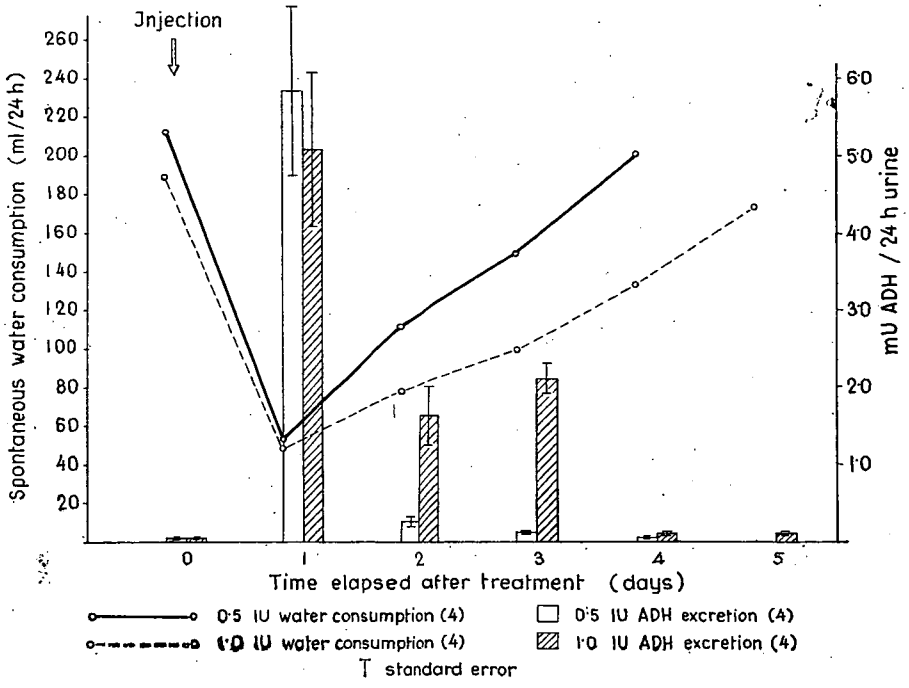


Fig. 23. Effect of Pitressin tannate on water intake and ADH excretion of rats with pituitary stalk lesion

the level of the non operated animals. At the study of the histological sections it was striking that the grafted neurohypophysis was significantly atrophied and its neurosecretory material had disappeared completely. These changes suggested the inactivity of the transplanted tissue. The sham-operated polyuric rats used as controls consumed somewhat less

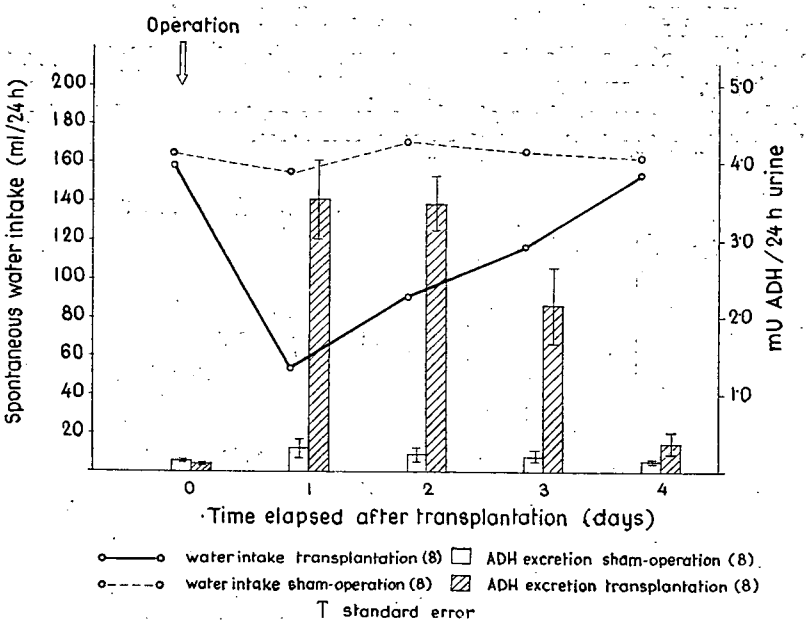


Fig. 24. Effect of transplantation of the neurohypophysis on water intake and ADH excretion of rats with pituitary stalk lesion

tap water after the operation, however, the alteration could not be considered to be significant. But it was remarkable that following the manipulation ADH excretion increased from 0,12 mU to 0,29 mU and this value decreased subsequently gradually.

A careful study of the results showed that there was a close correlation between the extent of the antidiuretic effect of each preparation and that of the urinary ADH activity. Our observations are summarized on Fig. 25. The rate of the ADH activity was determined in the following manner: the curve of the daily water intake was plotted in the way described in the case of the preceding figures, the starting point and the end point reaching this level were connected by a straight line and the area of the triangle obtained was expressed in cm^2 by means of a planimeter. These values composed the ordinate, whereas the total amount of ADH excreted in this period was plotted on the absciss. It may be seen that there is a pronounced correlation between the two parameters.

The experimental data revealed that the effect of the vasopressin preparations used by us was not only dose-dependent, but that the duration of the effect was also an important factor. Whereas, a larger dose of

purified lysine vasopressin did not influence the water intake of the polydipsic rats, a smaller quantity added to gelatine decreased the water consumption visibly. However, the change was the most marked following administration of the so-called long acting preparations (Pitressin tannate). But for that of 0,1 I. U. of lysine vasopressin, the recovery of all the preparations from the urine did not exceed 1 per cent. The amount of the ADH recovered from the urine did not depend upon the dose applied; the longer the time of the effect of the vasopressin used the higher the urinary ADH activity. Following neurohypophysial transplantation the water intake of the rats changed in a similar manner as it occurred in the oliguric interphase following lesion of the pituitary stalk.

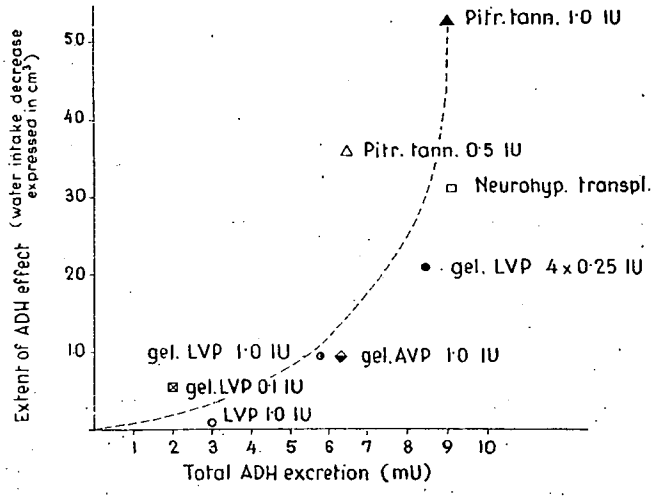


Fig. 25. Effect of various antidiuretic hormone preparations on water intake and total ADH excretion of rats with pituitary stalk lesion

It is known that vasopressin administered iv disappears rapidly from the blood (O'Connor, 1951; Dicker and Nunn, 1957; Heller and Zaidi, 1957). Daily examinations showed that only preparations with long lasting effect influenced appreciably the water metabolism. This is proved by the fact that 1,0 I. U. of lysine vasopressin did not reduce the 24 hours water consumption, if, on the other hand, gelatine was used to delay absorption, or the same dose was divided and administered in portions to the animals suffering from diabetes insipidus, the effect was far more pronounced.

The literature does not adopt an unequivocal view concerning the observations obtained by the comparison of the effect of lysine vasopressin and arginine vasopressin. According to Van Dyke, Engel and Adamson (1956) in dogs the antidiuretic effect of the two hormones was not identical. Munnick et al. (1958) found the same phenomenon in pigs. Sawyer (1958), on the other hand, showed that iv lysine vasopressin induced a more pronounced antidiuresis in rats than the same amount of arginine vasopressin, however, the duration of the effect was shorter. In our experiments the two ADH preparations caused the same changes in the water metabo-

lism. Recently BERDE (1963) dealt with pharmacological studies of neurohypophysial preparations.

Reports on the urinary excretion of ADH in humans (CLINE et al. 1953; NOBLE and TAYLOR, 1953; EISEN and LEWIS, 1954), dogs (HARE, HICKEY and HARE, 1941; O'CONNOR, 1951; THORN and SMITH, 1965), rabbits (HELLER, 1937) and rats (GINSBURG and HELLER, 1953; HELLER and ZAIDI, 1957; SAWYER, 1958) are relatively scarce. In the rat smaller amounts of ADH are recovered from the urine after administration of the hormone than in humans and dogs. GINSBURG and HELLER (1953) in rats found that 6,7 per cent of the injected vasopressin appeared in the urine and later HELLER and ZAIDI (1957) reported that 3 per cent of the octapeptide was excreted in the urine. The present experiments showed that only the rats treated with small doses of polypeptide (0,1 I. U. of lysine vasopressin in gelatine, 0,5 I. U. of Pitressin tannate) excreted more than 1 per cent of the hormone administered. The discrepancy between the data of the literature and the present values might have been due to the partial inactivation of the ADH content of the urine during the long period of collection (24 hours) occurring in spite of the application of procedures attempting to inhibit it (pH control, chilling). On the other hand, this could also have been a result of the fact that the conditions for our experiments were most adequate, i. e. polyuric animals without ADH supplies were used, whereas almost all other investigators observed rats having ADH supplies of their own. Finally, the discrepancy can also be explained by the difference that we employed larger amounts of vasopressin in our experiments than HELLER and his coworkers (GINSBURG and HELLER, 1953; HELLER and ZAIDI, 1957). Between the amounts of the ADH used and the percentage of its recovery from the urine, there was not a straight correlation; when smaller doses of vasopressin were injected the extent of the ADH activity of the urine was relatively marked. HELLER (1937) made similar observations in rabbits and concluded that within certain limits the quantity of ADH excreted by the kidney was independent of the concentration of the hormone in the blood. O'CONNOR (1951) and later THORN and SMITH (1965) showed that the adiuretin content of the urine was not related with the amount administered. Obviously, this may be in correlation with the normal limit of renal function expressed in the vasopressin-clearance (GINSBURG and HELLER, 1953; GINSBURG, 1957; HELLER and ZAIDI, 1957; SAWYER, 1958).

A comparison of the effect of retard vasopressin preparations and the changes occurring in the normal interphase indicated that in the former case the maximal ADH excretion coincided with that of the antidiuretic period, whereas this correlation could not be found in the oliguric period caused by the release of endogenous adiuretin. — According to the above suggestion this divergence could be due in the first place to the difference of the speed with which the ADH was released into the blood stream from the subcutaneous tissue and from the hypothalamo-neurohypophysial system impaired after destruction of the stalk.

Another interesting observation was that during the relative antidiuresis induced either by surgery or the administration of ADH preparations the output of the antidiuretic hormone exceeded the normal level to a great extent, yet the water intake and the urinary output were 2—3 times higher

than the values of the intact rats. These examinations cannot explain this problem; presumably the susceptibility of the kidney of polyuric animals to ADH decreased, or owing to the destruction the hypothalamic structures playing a part in the regulation of the water intake might have been damaged.

The observation that by the transplantation of neurohypophysial tissue under the kidney capsule a state resembling to the normal oliguric interphase could be induced in polyuric rats seemed interesting and this furnished new data concerning the pathogenesis of the antidiuretic period. HOLLINSHEAD (1964) made similar observations in dogs. According to this author the phenomenon of the interphase could be explained by the effect of ADH released from the injured neural lobe. However, it has not been elucidated, why the antidiuresis only developed on the third or fourth day after the lesion, while spontaneous water consumption and urinary output decreased immediately following neurohypophysial transplantation. A certain inconsistency could be shown by comparing the extent of urinary ADH secretion and the change in the water intake. As following neurohypophysial transplantation more than the double amount of the hormone was excreted than after stalk lesion, the question arises: whether the site of the transplantation was well chosen, knowing that the hormone acts on the kidney and that the renal tissue plays an important role in the inactivation of ADH (GINSBURG and HELLER, 1953; HELLER and ZAIDI, 1957; HANKISS et al. 1961; HANKISS, 1963). It would not have been better to place the neurohypophysis under the capsule of the spleen considering that the blood of the spleen flows through the liver inactivating vasopressin, as well (HELLER and ZAIDI, 1957; HANKISS et al. 1961). On the other hand, according to our method it can be conceived that a part of the ADH released from the neurohypophysial graft did not gain access to the systemic circulation and that not the whole amount of the hormone got into contact with the inactivating system, but reached directly the renal tubules by diffusion. This would explain the enhanced ADH excretion; however, further experiments are needed to prove this assumption.

The urinary ADH content of the sham-operated rats suffering from diabetes insipidus used as controls for the group with grafted neurohypophysis also rose after the operation and approached normal levels. It is known since a long time that following surgical manipulations ADH mobilisation increases (LE QUESNE and LEWIS, 1953; DUDLEY et al. 1954; LE QUESNE, 1954; HAYES et al. 1957; MORAN et al. 1964) and even in neurohypophysectomized rats possessing a reduced hormone supply a certain ADH response could be demonstrated after intensive noxious stimuli (MOLL and DE WIED, 1962). Previously we also demonstrated that the vasopressin store of stalk lesioned animals was not completely exhausted, and in some cases some „regression" could also be observed (see later). At any rate, the slight reduction in the water intake and urinary output showed that the quantity of the released hormone was not considerable and exerted its effect only for a relatively short time.

Chapter IV.

EXAMINATION OF THE WATER METABOLISM IN RATS SEVERAL WEEKS AFTER DESTRUCTION OF THE PITUITARY STALK

(Experiments on the „regression" of diabetes insipidus)

In previous experiments concerned with the hypothalamo-hypophysial relations of water metabolism (see Chapter III.) it was found that in rats with stalk lesion, obviously as a result of damage to the tissues producing the antidiuretic hormone, a characteristic disturbance of the water metabolism: diabetes insipidus developed. However, when water loads in series were applied diuresis gradually diminished after a few weeks in most animals and one or two months after operation it did not exceed, or hardly exceeded the mean values obtained in the non-operated control animals. When oral tap water loads were applied for the examinations, the results seemed to indicate that in most of the rats destruction of the pituitary stalk failed to produce persistent diabetes insipidus.

Other authors also found that in the rat, operation in the hypophysial region may be followed by a regression of diabetes insipidus. For example it has been shown by CHESTER JONES (1957) that spontaneous water intake was merely temporarily increased in some of the neurohypophysectomized rats. ALEXANDER (1958) induced hypothalamic lesions in several hundred rats and observed that the polyuria following operation was not always permanent; chronic diabetes insipidus developed in merely one-third of the animals showing considerable polyuria during the period immediately following operation. RANDALL et al. (1960) and SHARKEY et al. (1961) have reported similar observations in humans.

Therefore, it seemed interesting to study on the one hand, the water metabolism of rats several weeks after the destruction of the pituitary stalk by methods other than that involving tap water loads and on the other, to investigate what mechanism might play a role in the regression of the polyuric reaction observable in response to oral tap water administration during the postoperative period.

In the present chapter the results of these experiments are described.

Albino rats of both sexes fed on a mixed diet weighing 150—260 g were used. The destruction of the pituitary stalk was performed partly in the way described in previous chapters by means of the Horsley—Clarke and partly by the modified Krieg apparatus. However, in each experimental series the same apparatus was used. Water metabolism was controlled several times after operation. In the experiments to be described only those rats were used which showed polyuria in response to the oral administration of tap water in the postoperative period and in which the polyuria ceased one month after destroying the stalk and diuresis no longer differed significantly from the average of that of the non-operated controls. These

two criteria were supplemented by a third one, the hypothalamic hypophysial system of the animals was studied histologically in serial sections. Only the changes in water metabolism of the animals in which the hypophysial stalk had been destroyed in its entire length (so-called total stalk lesion) were taken into consideration.

The animals not meeting the above criteria, those in which the stalk had not, or only partially been damaged, or in which no disturbance of water metabolism had developed after operation, were not included in the evaluation. (It should be noted that total stalk lesion invariably resulted in polyuria.) In a few rats with total stalk lesion the diabetes insipidus-like condition did not cease after 2—3 months; these animals continued to respond with polyuria to the oral administration of tap water. Since our aim was to study the regression of diabetes insipidus and the mechanism of the process, these animals, too, were excluded from the evaluation, however, for the sake of comparison they were used in some cases as rats suffering from diabetes insipidus, but this was always denoted on the Tables.

The „water loading method" used to prove the disturbance of the water metabolism, the procedure to determine the localisation and extent of the lesion and the details of the histological technique were already described previously. It should be remarked that the diuretic reaction of the animals was evaluated as a „summation urine output" after the oral administration of 5 per cent/body weight of tap water. This value indicated the intensity of diuresis and is the area under the summation polygon of the cumulative urine outputs measured at 1 hour intervals for 8 hours multiplied by the reciprocal of the amount of water administered. The rats were killed by decapitation, the hypothalamus was removed together with the pituitary fixed in Susa-solution, embedded in paraffin and cut up serially into 12 micron thick sections at 100 micron distances. The sections cut in the frontal plane were stained with haematoxylin-eosin, in some cases with Gomori's chromalum-haematoxylin, Gomori's aldehyde-fuchsin, as well as with Paget-Eccleston's aldehyde-thionin.

We studied several aspects of the water metabolism of rats in which the stalk had been destroyed one month previously. Following the oral administration of tap water we determined the electrolyte- and creatinine excretion, the specific gravity of the urine, the glomerular filtration rate, spontaneous water intake and water excretion, the effect of cortisone, physiological NaCl, and ethanol on the diuretic reaction, the morphology of the hypothalamus and neurohypophysis, the ADH excretion and the ADH content of the neurohypophysis and the hypothalamus. Finally some manipulations were applied anticipating that they will induce a release of adiuretin. The results obtained were compared with those yielded by non-operated controls, or if it seemed necessary, with those obtained in rats submitted to stalk lesions a few days before in which polyuria had developed.

Table 10.

Effect of various antidiuretic hormone preparations on water intake and total ADH excretion of rats with pituitary stalk lesion

Group		Time elapsed after operation	Fluid administered	No. of animals	Body weight g	Amount of urine excreted in 5 hours calculated for 100 g body weight ml	Concentration (m Eq/l)			Amount of urine excreted in 5hours calculated for 100 g/body weight (μ Eq/100 g body weight)			Na/K ratio	Amount of creatinine excreted in 5 hours calculated for 100 g body weight (mg) 100 g body weight
							Na	K	Cl	Na	K	Cl		
I.	Intact	—	Tap water	10	162,5 ±2,3*	4,9 ±0,1	29,8 ±4,4	14,8 ±2,3	32,6 ±5,5	148,1 ±22,5	69,4 ±11,2	163,8 ±27,6	2,1 ±0,2	0,44 ±0,03
II.	Stalk lesion	6—8 days	Tap water	10	191,5 ±7,9	9,6 ±0,6	15,2 ±1,1	7,0 ±0,4	16,5 ±2,1	145,3 ±18,7	66,0 ±4,6	155,5 ±22,0	2,2 ±0,2	0,54 ±0,06
III.	Stalk lesion	More than 1 month	Tap water	10	81,25 ±5,0	4,7 ±0,2	13,5 ±2,0	6,7 ±0,5	10,5 ±1,3	62,0 ±8,8	31,9 ±3,2	47,9 ±5,4	1,8 ±0,3	0,34 ±0,04
IV.	Intact	—	Physiological Na Cl	10	176,0 ±2,7	3,9 ±0,2	151,9 ±4,5	27,2 ±2,2	184,2 ±5,6	585,6 ±33,5	105,4 6,5	702,9 ±33,8	4,9 ±0,5	0,48 ±0,08
V.	Stalk lesion	6—8 days	Physiological Na Cl	10	208,0 ±9,9	10,9 ±0,5	70,4 ±6,7	11,7 ±1,4	79,6 ±5,7	723,7 ±56,0	122,8 ±12,4	839,1 ±52,1	6,3 ±0,5	0,56 ±0,08
VI.	Stalk lesion	More than 1 month	Physiological Na Cl	10	186,0 ±4,6	9,9 ±0,7	82,1 ±8,5	11,4 ±1,2	87,4 ±5,8	758,4 ±30,5	110,1 ±13,2	874,4 ±51,8	7,8 ±0,9	0,47 ±0,06

* Standard error

Probability:									
	I/II.	$p \ll 0,001$	$0,02 > p > 0,01$	$p < 0,01$	$0,02 > p > 0,01$	$p > 0,05$	$p > 0,05$	$p > 0,05$	$p > 0,05$	$p > 0,05$
	I/III.	$p > 0,05$	$p < 0,01$	$p < 0,01$	$p < 0,001$	$p < 0,01$	$p < 0,01$	$p < 0,01$	$p > 0,05$	$p > 0,05$
	I/IV.	$p < 0,01$	$p \ll 0,001$	$p < 0,01$	$p \ll 0,001$	$p \ll 0,001$	$0,02 > p > 0,01$	$p \ll 0,01$	$p < 0,001$	$p > 0,05$
	II/III.	$p < 0,001$	$p > 0,05$	$p > 0,05$	$0,05 > p > 0,02$	$p < 0,001$	$p < 0,001$	$p < 0,01$	$p > 0,05$	$0,02 > p > 0,01$
	II/V.	$p > 0,05$	$p \ll 0,001$	$p < 0,01$	$p \ll 0,001$	$p \ll 0,001$	$p < 0,001$	$p \ll 0,01$	$p < 0,001$	$p > 0,05$
	III/IV.	$p > 0,05$	$p \ll 0,001$	$p \ll 0,001$	$p \ll 0,001$	$p \ll 0,001$	$p \ll 0,001$	$p \ll 0,001$	$p < 0,001$	$p > 0,05$
	IV/V.	$p \ll 0,001$	$p < 0,001$	$p < 0,001$	$p < 0,001$	$0,05 > p > 0,02$	$p > 0,05$	$0,05 > p > 0,02$	$p > 0,05$	$p > 0,05$
	IV/VI.	$p < 0,001$	$p \ll 0,001$	$p < 0,001$	$p < 0,01$	$p < 0,01$	$p > 0,05$	$0,02 > p > 0,01$	$0,02 > p > 0,01$	$p > 0,05$
	V/VI.	$p > 0,05$	$p > 0,05$	$p > 0,05$	$p > 0,05$	$p > 0,05$	$p > 0,05$	$p > 0,05$	$p > 0,05$	$p > 0,05$

1. Urinary excretion of sodium, potassium, chloride and creatinine

The animals had been fasted for ten hours before the experiment, water had been allowed ad libitum. At the beginning of the experiment the drinking water was withheld, the animals were placed one by one into cages suitable for collecting urine and were given by stomach tube tap water or physiological NaCl solution, in amounts of 5 per cent/body weight. The duration of the experiment was 5 hours. By the end of the 5th hour the urine volume was measured with an accuracy of 0,1 ml and samples of urine were tested for Na, K, Cl and creatinine concentration. Na and K were determined by flame photometry, Cl by the method of SCHALES and SCHALES (1941), creatinine by the method of FOLIN-WU (1919) as modified by BROD and SIROTA (1948).

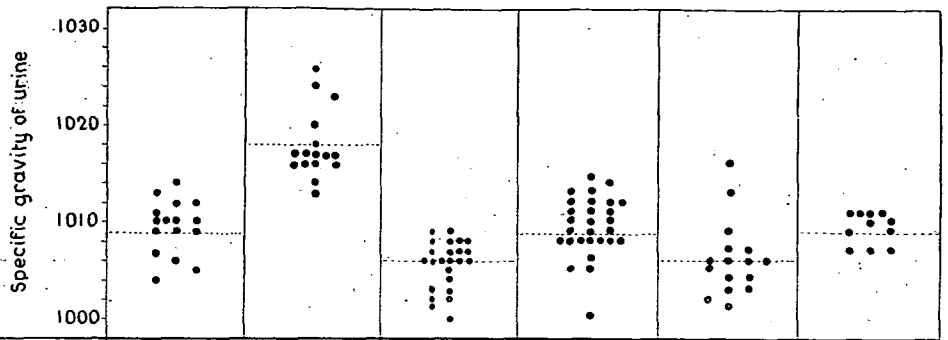
The volume of urine was computed for 100 g body weight. The values of sodium, potassium and chloride were expressed in mEqu/1 and the total amounth excreted in 5 hours, computed for 100 g body weight in μ-Equ. We determined the creatinine values expressed in mg creatinine output in 5 hours computed for 100 g body weight. Finally the ratio of sodium and potassium (Na/K) was also estimated.

The results for electrolyte and creatinine excretion are presented in Table 10. The Table shows that the average urine output of the rats in which the stalk had been destroyed one week previously following the oral administration of tap water did not substantially differ from that of the non-operated controls. However, as compared to the corresponding control values, urinary Na, K, Cl concentration and the amount of electrolyte output were significantly lower. The Na/K ratio did not change, whereas creatinine extretion slightly decreased. Table 10. also contains data of rats which had been subjected to stalk lesion a few days earlier. These animals excreted significantly more urine. The concentrations of Na, K and Cl decreased, the total electrolyte output was practically unchanged and the creatinine output was slightly increased.

The rats subjected to stalk lesion several weeks earlier responded to the oral administration of physiological saline solution with marked polyuria. The values were almost identical with, or slightly different from, those for the rats in which the stalk had been destroyed a few days earlier. Urine output increased in both groups, Na, K and Cl concentrations decreased, K and creatinine outputs were practically unchanged, whereas Na and Cl excretion was slightly increased. A comparison of the data for the tap water loaded animals with those for the physiological saline loaded ones shows that following the administration of saline the output not only of Na and Cl, but also of K increased. It is remarkable that hyperpotassuria also occurred in animals in which the stalk had been destroyed a month previously like in those in which the experiment was performed in the immediate postoperative state.

2. *Specific gravity of the urine*

The specific gravity of the urine was determined by the piknometric method of GÁL et al. (1953), simultaneously with the urinary volume 5 hours after the animals had been given 5 per cent/body weight of tap water or physiological saline orally. The results are shown in Fig. 26. The non-



Group	Intact		Stalk lesion			
Fluid administered orally	Tap water	phys. NaCl	Tap water	phys. NaCl	Tap water	phys. NaCl
Time elapsed after operation (days)	—	—	6 - 14	6 - 14	More than 30 days	More than 30 days
Number of animals	16	16	24	28	16	11
Body weight g	190 ± 3·7 *	188·9 ± 12·8	187·5 ± 3·8	180·7 ± 3·0	171·6 ± 5·1	182·3 ± 5·9
5 hours urine output (ml) calculated for 100 body weight	4·3 ± 0·1	2·2 ± 0·5	8·9 ± 0·5	9·5 ± 0·3	4·3 ± 0·4	8·4 ± 0·4
Specific gravity of urine	1009 ± 0·9	1018 ± 0·8	1006 ± 0·5	1009 ± 0·4	1006 ± 1·0	1009 ± 0·5

* Standard error

Fig. 26. Specific gravity of urine in intact rats and in rats with pituitary stalk lesion following oral administration of tap water and physiological NaCl

operated rats loaded with physiological saline excreted less urine of higher specific gravity than did the intact animals after water loading. A few days after the destruction of the pituitary stalk both groups excreted ample amounts of urine of lower specific gravity. (There was no marked difference in specific gravity of the urine between intact rats and rats with hypophysial stalk lesion after the water load. This was not surprising since the non operated animals had also been treated with tap water. There was more pronounced difference between the two groups on treatment with physiological saline. (The specific gravity of the urine was low in the group subjected to stalk lesion several weeks earlier after both water and saline loads. Urine output by the animals treated with tap water was comparable to that of the non operated controls, whereas in response to saline, intensive polyuria developed.)

3. *Glomerular filtration*

Glomerular filtration rate was determined by the exogenous creatinine clearance method of LOTSPEICH (1949). Rats in which the stalk had been destroyed several weeks earlier and intact rats were used. One group of the rats with stalk lesion was treated with subcutaneous doses of cortisone (Adreson, Organon, Oss, 10 mg/rat) for four days. The results are shown in Table 11. Our data indicate that in this series minute diuresis and exogenous

Table 11.

Exogenous creatinine clearance and minute diuresis in intact rats and in rats with pituitary stalk lesion

Group		Time elapsed after-operation	No. of animals	Body weight g	Exogenous creatinine clearance ml/100 cm²/min	Amount of urine ml/100 cm²/min
I.	Intact	—	11	168,2±8,9*	0,709±0,076	0,030±0,002
II.	Stalk lesion	More than 30 days	12	185,8±5,7	0,438±0,050	0,018±0,002
III.	Stalk lesion + cortisone	More than 30 days	7	152,1±3,2	0,703±0,078	0,028±0,002
* Standard error				I/II.	$p<0,01$	$p<0,001$
			Probability:	I/III	$p>0,05$	$p>0,05$
				II/III.	$p<0,01$	$p<0,01$

creatinine clearance rate significantly decreased in the animals in which the stalk had been destroyed several weeks before. These changes were abolished by cortisone treatment; the minute diuresis of the animals submitted to stalk lesion several weeks previously and the exogenous creatinine clearance was also under these conditions on the level of that of the non-operated animals.

4. *Spontaneous water consumption and urinary output*

In an attempt to study the nature of the disturbance of the water metabolism observed in rats submitted to stalk lesion several weeks earlier we have examined the 24-hour spontaneous water intake and urinary output and compared it with that of the non-operated controls, that of those operated 1—2 weeks before and that of permanently polyuric stalk lesioned animals. The results are presented in Table 12. They indicate that

1—2 weeks after the stalk had been destroyed water consumption significantly increased. However, water intake was increased also in the rats stalk lesioned several weeks before and suffering from permanent diabetes insipidus. What more, the rats submitted to stalk lesion some weeks previously in which following oral water loads polyuria no longer developed drank twice as much water than the controls. The values of the 24 hours urinary output changed in each animal parallel to those of the water intake.

5. *Effect of cortisone, physiological saline and ethanol on the summation urine output*

As previous investigations had indicated that in most rats submitted to stalk lasion oral tap water administration induced only transient polyuria

Table 12.

Daily spontaneous water intake and urine output of intact rats and rats with pituitary stalk lesion

	Group	Time elapsed after operation	No of animals	Body weight g	Water intake ml/24 h	Urine output ml/24 h
I.	Intact	—	8	196,0±6,1*	27,0± 1,6	13.6±1,0
II.	Stalk lesion	6—14 days	8	208,5±12,2	212,4±14,6	169,0±12,8
III.	Stalk lesion (permanent diabetes insipidus)	More than 1 month	8	254,5±11,2	166,9±18,8	128,8±16,2
IV.	Stalk lesion (regression of diabetes insipidus)	More than 1 month	8	261,0±9,3	56,2± 4,1	23,9±2,1
				I/II.	$p \ll 0{,}001$	$p \ll 0{,}001$
				I/III.	$p < 0{,}001$	$p < 0{,}001$
				I/IV.	$p < 0{,}001$	$p < 0{,}001$
			Probability:	II/III.	$p > 0{,}05$	$p > 0{,}05$
				II/IV.	$p \ll 0{,}001$	$p \ll 0{,}001$
				III/IV.	$p < 0{,}001$	$p < 0{,}001$

* Standard error

Table 13.

Effect of cortisone, phys. NaCl or ethanol on summation urine output of intact rats and rats with pituitary stalk lesion operated more than 1 month previously

	Group	Fluid administered orally	Treatment	No. of animals	Body weight g	Summation urine output	Probability	
I.	Intact	tap water	—	10	185,0 ±5,7*	6,34 ±0,22	I/II.	$p<0,01$
II.	Intact	tap water	cortisone	10	167,0 ±3,1	7,67 ±0,27	I/III.	$p<0,001$
III.	Intact	phys. NaCl	—	10	208,5 ±7,7	3,13 ±0,36	I/IV.	$p<0,001$
IV.	Intact	Ethanol	—	10	180,0 ±5,5	8,20 ±0,22	V/VI.	$p<0,001$
V.	Stalk lesion	tap water	—	10	194,0 ±7,7	6,56 ±0,37	V/VII.	$p<0,001$
VI.	Stalk lesion	tap water	cortisone	10	160,0 ±4,9	9,64 ±0,63	V/VIII.	$p<0,01$
VII.	Stalk lesion	phys. NaCl	—	10	185,0 ±4,3	11,68 ±0,87	I/V.	$p>0,05$
VIII.	Stalk lesion	Ethanol	—	10	180,0 ±5,2	8,02 ±0,29	II/VI.	$p<0,01$
							III/VII.	$p\ll0,001$
							IV/VIII.	$p>0,05$

*Standard error

following surgery and that after 1—2 months had elapsed the diuresis returned to the normal level, it seemed interesting to examine the influence exerted by different substances on the water metabolism of rats in which the stalk had been destroyed in the preceding weeks. One group of such rats was given 5 per cent/body weight of tap water by mouth and treated for 2—3 days with daily doses of 10 mg/rat of cortisone (Adreson, Organon Oss) subcutaneously. A second and a third group was given 5 per cent/body weight of physiological NaCl solution or 5 per cent ethanol, instead of tap water. Each rat was placed in a metabolic cage, urine output was measured every hour for 8 hours and the urine excreted was evaluated as „summation urine output". The results are presented in Table 13. The data indicate that the summation urine output of the rats submitted to stalk lesion several weeks earlier did not differ significantly from that of the non-operated controls following oral tap water treatment. On the other hand, treatment with cortisone or oral administration of physiological NaCl solution resulted in significant polyuria, whereas the oral administration of ethanol only caused moderate polyuria.

6. Morphology of the hypothalamus and neurohypophysis

The hypothalamo-neurohypophysial system of the rats submitted to stalk lesion several weeks earlier showed characteristic morphological changes. (The changes found in the anterior pituitary and the pars intermedia are discussed elsewhere. As to the hypothalamus, we shall only deal with changes in the supraoptic nucleus, as well as with those in the magnocellular portion of the paraventricular nucleus.) No quantitative histological methods (counting of ganglion cells, karyometry) were applied at the examination of the supraoptic nucleus and the paraventricular nucleus (the magnocellular portion) but it seemed as if the number of ganglion cells was lower than normal and their size smaller than in the intact animals. The neurohypophysis showed marked atrophy, its size was

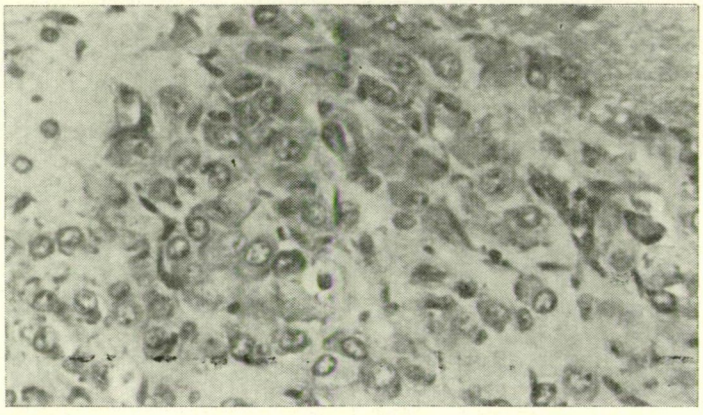


Fig. 27. Nucleus supraopticus of control rat. Gomori-chromalum-haematoxylin. 278 x

significantly diminished. Beside the atrophy of the hypothalamus and neurohypophysis it was most conspicuous that the neurosecretory material staining well with Gomori's chromalum-haematoxylin, Gomori's aldehyde-fuchsin, as well as Paget—Eccleston's aldehyde-thionin procedures, had almost completely disappeared. In the magnocellular nuclei of the anterior hypothalamus, as well as in the neurohypophysis there were only occasionally minute granules staining with the above procedures (Fig. 27, 28) The destroyed hypophysial stalk showed no signs of regeneration; no new posterior pituitary-like structure developed.

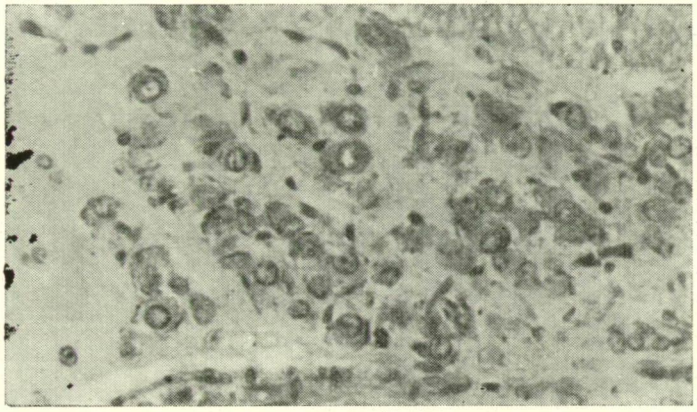


Fig. 28. Nucleus supraopticus of rat with stalk lesion 1 month previously. Atrophy can be seen. Gomori-chromalum-haematoxylin. 278 x

7. *ADH content of the hypothalamus and posterior pituitary; ADH excretion*

The determination of the antidiuretic hormone was performed in rats under ethanol anaesthesia as described in the previous Chapter (De Wied, 1960). Extracts were prepared from the hypothalamus (total hypothalamus) and the neurohypophysis with physiological NaCl solution. The samples obtained from urine collected for 24 hours were adjusted to pH 7 by 0,1 N HCl and made isotonic by addition of various amounts of saline. These procedures were also described previously. As controls, intact and rats suffering from chronic diabetes insipidus were used. The results were presented on Table 14. The Table shows that the weight of the neurohypophysis decreased identically in both operated groups. However, the ADH content of the posterior pituitary was higher in the group than in animals, in which the polyuria was consistent. The difference is still more substantial concerning the amount of ADH contained in the hypothalamus, as in the rats, in which the diabetes insipidus diminished considerably, the ADH content of the hypothalamus approached that of the non-operated controls. On studying the hormone output it was found that the urine of poly-

Table 14.

ADH secretion, weight of neurohypophysis, ADH content of hypopthalamo-neurohypophysial syste m of intact rats and rats with stalk lesion operated more than 1 month previously

	Group	No. of animals	Body weight g	ADH secretion mU/24 h urine	Weight of neurohypophysis mg	ADH content of neurohypo-physis mU	ADH content of hypothalamus mU
I.	Intact	8	196,0± 6,1*	0,47±0,060	0,90±0,046	1123,7±49,4	112,6±13,5
II.	Stalk lesion	8	254,5±11,2	0,05±0,013	0,43±0,027	17,7± 3,7	12,5± 1,6
III.	Stalk lesion	8	261,0± 9,3	0,22±0,052	0,48±0,037	90,7± 6,9	73,4± 8,3
			I/II.	$p<0{,}001$	$p\ll0{,}001$	$p\ll0{,}001$	$p<0{,}001$
Probability:			I/III.	$p<0{,}01$	$p<0{,}001$	$p\ll0{,}001$	$0{,}02>p>0{,}01$
			II/III.	$p<0{,}01$	$p>0{,}05$	$p\ll0{,}001$	$p<0{,}001$

* Standard error

uric animals only contained a minimal antidiuretic activity, whereas the ADH secretion was higher in the group in which there was a regression of the diabetes insipidus.

8. Effect of acethylcholine on the diuretic reaction

The above results suggest that the restoration of the antidiuretic hormone production might have played some role in the normalization of the diuretic reaction of the animals submitted to stalk lesion in the preceding weeks. To study this question the application of stimuli mobilising ADH in normal rats seemed the most adequate. For this purpose acethylcholine was used in the following experimental series. According to some data (PICKFORD, 1939; 1947; PICKFORD and WATT, 1951; ABRAHAMS and PICKFORD, 1954; CASENTINI et al. 1957; KIVALO and ARKO, 1957; KOVÁCS et al. 1959) this mediator substance causes inhibition of the diuresis as the result of the release of ADH.

The method used for the examination of the diuretic reaction has already been described. At the beginning of the experiment the tap water was withheld and each animal was placed in a metabolic cage, subsequently tap water was given orally by a stomach tube 5 per cent/body weight. After two hours the quantity of excreted urine was measured and the water loads (5 per cent/body weight) were repeated as described above. From this period on the amount of the urine was measured with an accuracy of 0,1 ml for 3 hours at intervals of 15 minutes: the amount of the urine excreted at different periods was expressed as the percentage of the water not excreted till the second water load (the quantity of the urine excreted before the second water load was subtracted from the sum of the two water loads). The results were evaluated biometrically by means of Student's „t" test.

The diuretic reaction of the rats with stalk lesion was examined 6—7 days or 4—6 weeks after the operation. In these periods in the first group the oliguric interphase had already ceased and in the second the diabetes insipidus-like state was over. Some of the rats were only used once, others, on the other hand, were employed both in the acute and chronic experiments.

The acethylcholine (VEB, Berlin) was administered intraperitoneally simultaneously with the second water load (1 mg/100 g body weight). The controls were given physiological NaCl (0,5 ml/100 g body weight) intraperitoneally in the course of the second water load, as well. It should be remarked that the acethylcholine was diluted so that 0,5 ml of the solution contained the amount which we wanted to administer (100 g/body weight). Some animal groups were pretraeated with cortisone. 10 mg of cortisone (Adreson, Organon, Oss) was applied for 3 days subcutaneously. The experiment was performed on the fourth day. Then the animals were given another dose of 10 mg cortisone during the first water load.

For the experiments to be reported 40 non-operated rats and 70 rats with total stalk lesion were used.

The summarized results are illustrated on Table 15. The Table indi-

cates that in intact rats following oral water loads a characteristic diuretic reaction developed; on the average 46 per cent of the water retained till the second water load was excreted by the animals in 60 minutes, and the amount of the urine continued to increase gradually. A characteristic inhibition of the diuresis could be observed in intact rats after acethylcholine treatment. The oliguria lasted on the average 90 minutes, then the urine output gradually increased and after 180 minutes, i. e. at the end of the experiment, it was practically the same as that of the controls.

When the examination was performed 6—7 days after the lesion of the stalk a pronounced diuretic reaction could be observed. It should be mentioned that the diuresis started somewhat less rapidly — in the 60 th minute it was slightly, but not significantly, less than the values of the non-operated controls — however, at the end of the experiments the animals as compared to the intact rats already excreted considerably more urine. After administration of acethylcholine an insignificant inhibition of the diuresis, only lasting for a short period, could be demonstrated in the animals submitted to stalk lesion 5—7 days previously, it was, however, far milder than that of the controls.

By 4—6 weeks after stalk lesion the diuretic reaction was without any treatment very protracted; even at the end of the experiment the quantity of the urine did not reach that of the controls — though it approached it. The possible effect of acethylcholine could thus not be evaluated adequately. Although, the amount of the urine of the animals injected with acethylcholine showed during the first 60 minutes a somewhat more pronounced decrease, in the untreated ones an inhibition of the diuresis could be observed as well. In our opinion, therefore, final conclusions cannot be drawn from this experiment.

In view of this fact experimental conditions had to be created to restore the diuretic reaction of the animals submitted to stalk lesion 4—6 weeks before. For this purpose administration of cortisone seemed the most suitable; it is known that following oral water loads this drug is capable to normalize the protracted urinary output of hypophysectomized animals (Boss et al. 1952; BRUNNER et al. 1956; KOVÁCS et al. 1958; DÁVID et al. 1959). The experiments supported this assumption completely; in the rats treated with cortisone and submitted to stalk lesion 4—6 weeks previously a marked diuretic reaction developed.

The cortisone pretreatment rendered it possible to study adequately the effect exerted by acethylcholine on the rats submitted to stalk lesion in the preceding 4—6 weeks. These examinations showed that in such animals acethylcholine only caused a mild inhibition of the diuresis. A comparison of the data of the animals treated with cortisone and submitted to stalk lesion 4—6 weeks earlier indicated that beyond doubt the amount of the urinary output slightly decreased in the first 60 minutes, but substantially less than in the group injected with acethylcholine in which the stalk had not been destroyed. (The cortisone pretreatment was also performed in intact animals. In the course of its administration an enhanced diuretic reaction was observed. Cortisone pretreatment did not influence appreciably the diuresis inhibiting effect of acethylcholine.)

Table 15.

Effect of acethylcholine on urinary output of intact rats and of rats with pituitary stalk lesion

No.	Group	Time elapsed after operation	Treatment	No. of animals	Boody weight g	Average urinary output expressed in per cents of the retained water				Probability				
						60'	90'	120'	180'		60'	90'	120'	180'
I.	Intact	—	—	10	174,0 ±1,2*	46,6 ±5,4	70,6 ±4,4	73,7 ±3,3	77,4 ±3,2	I/II.	$p<0{,}001$	$p\ll0{,}001$	$0{,}05>p>0{,}02$	$p>0{,}05$
										I/III.	$p>0{,}05$	$p>0{,}05$	$p>0{,}05$	$p<0{,}01$
II.	Intact	—	Acethly-choline	10	189,5 ±9,0	5,1 ±1,4	29,0 ±3,6	62,1 ±4,4	70,4 ±5,2	I/IV.	$p<0{,}001$	$0{,}05>p>0{,}02$	$p>0{,}05$	$p<0{,}001$
III.	Stalk lesion	6—7 days	—	12	178,3 ±4,8	33,1 ±7,3	59,0 ±4,6	84,2 ±8,5	135,7 ±13,7	I/V.	$p<0{,}001$	$p<0{,}001$	$p<0{,}001$	$0{,}02>p>0{,}01$
										I/VI.	$p\ll0{,}001$	$p<0{,}001$	$p<0{,}01$	$p>0{,}05$
IV.	Stalk lesion	6—7 days	Acethyl-choline	12	187,1 ±5,5	17,4 ±5,2	55,4 ±5,3	86,2 ±7,1	146,0 ±8,6	I/VII.	$p>0{,}05$	$p>0{,}05$	$p>0{,}05$	$0{,}02>p>0{,}01$
V.	Stalk lesion	4—6 weeks	—	17	177,4 ±4,7	16,2 ±4,7	34,6 ±4,6	43,1 ±5,2	64,3 ±3,4	I/VIII.	$0{,}02>p>0{,}01$	$0{,}05>p>0{,}02$	$p>0{,}05$	$p>0{,}05$
										I/IX.	$p<0{,}001$	$p>0{,}05$	$0{,}02>p>0{,}01$	$0{,}02>p>0{,}01$
VI.	Stalk lesion	4—6 weeks	Acethyl-choline	23	176,7 ±3,6	8,8 ±2,3	27,9 ±3,7	49,6 ±5,1	72,0 ±6,2	I/X.	$p<0{,}001$	$p<0{,}001$	$p<0{,}01$	$0{,}05>p>0{,}02$
VII.	Stalk lesion	4—6 weeks	Cortisone	14	169,3 ±5,0	48,6 ±5,6	66,7 ±4,6	72,7 ±5,6	95,2 ±5'1	II/X.	$p>0{,}05$	$p>0{,}05$	$p>0{,}05$	$p>0{,}05$
										III/IV.	$p>0{,}05$	$p>0{,}05$	$p>0{,}05$	$p>0{,}05$
VIII.	Stalk lesion	4—6 weeks	Cortisone acethyl-choline	14	168,2 ±5,5	26,5 ±5,3	56,8 ±4,4	69,5 ±6,0	92,7 ±6,9	V/VI.	$p>0{,}05$	$p>0{,}05$	$p>0{,}05$	$p>0{,}05$
IX.	Intact	—	Cortisone	10	187,5 ±6,8	78,2 ±3,1	79,7 ±3,0	85,8 ±3,2	99,6 ±7,3	V/VII.	$p<0{,}001$	$p<0{,}001$	$p<0{,}001$	$p<0{,}001$
										VI/VIII.	$p<0{,}01$	$p<0{,}001$	$0{,}02>p>0{,}01$	$0{,}05>p>0{,}02$
X.	Intact	—	Cortisone + acethyl-choline	10	171,0 ±4,8	9,1 ±2,3	31,8 ±3,0	56,6 ±3,1	68,0 ±2,3	VII/VIII.	$p<0{,}01$	$p>0{,}05$	$p>0{,}05$	$p>0{,}05$

*Standard error

9. *Effect of hyperosmosis on ADH output*

Subsequently the effect of hyperosmosis was studied. Hyperosmosis was induced in the manner described in the previous chapter with a 15 per cent solution of NaCl. The ADH determination of the urine collected during 6 hours before and after the treatment were carried out according to De Wied (1960). The results were compared with the values of the non-operated controls and those of persistently polyuric rats.

Table 16. shows that in the control period the amount of the urine was significantly increased in rats suffering from permanent diabetes insipidus, whereas that of the moderately polyuric animals was only slightly enhanced. Following the injection the urinary output exhibited a rise in the control group, in the second group a marked diminution occurred, in the third one there was no change. On examining the ADH excretion it could be established that following administration of a hypertonic NaCl solution the non-operated controls excreted substantially more adiuretin. In the consistently polyuric animals this change was insignificant. The rats suffering only from a moderate diabetes insipidus possessed a certain ADH capacity which is proved by the fact that following hyperosmosis hormone-mobilization developed and the urinary ADH excretion increased. This change was not so pronounced as in the controls, however, it exceeded the values measured in the same way in the case of rats suffering from acute or chronic manifest diabetes insipidus.

The results suggest that the disturbance of water metabolism which develops in conjunction with the lesion of the pituitary stalk undergoes a gradual alteration. Following operation (except for the phase of temporary oliguria beginning on the third day and lasting 1—2 days) the animals responded with marked polyuria to the oral administration of tap water, whereas this polyuric reaction was no longer demonstrable in the 1—2 postoperative months, when diuresis did not exceed or only slightly exceeded the average for the non-operated controls in the majority of the animals, so that one might have felt tempted to claim that the diabetes insipidus-like condition had ceased. However, the normalization of water metabolism was only virtual because the spontaneous water intake continued to exceed that of the controls and the specific gravity and electrolyte concentration of the urine were lower, furthermore the diuretic reaction was also enhanced after cortisone treatment and following administration of 0,9 per cent solution of NaCl instead of tap water the rats were markedly polyuric.

It was remarkable that the two adequate methods for studying water metabolism (the diuretic reaction in response to the oral odministration of water and spontaneous water consumption) yielded different results in the rats submitted to stalk lesion several weeks earlier; the animals showing a normal diuretic response had an enhanced water intake. Similar conclusions had been drawn form previous experiments concerning the water metabolism of hypophysectomized rats (Kovács, 1963). These animals showed excessive antidiuresis in response to an oral water load, while spontaneous water intake did not decrease. It seemed therefore, that the changes in the diuretic reaction in response to an oral water load and those

Table 16.

Effect of hyperosmosis on urinary output and ADH secretion of nonoperated controls and rats with pituitary stalk lesion operated a month previously

Group		No. of animals	Amount of urine ml/ hours		Secretion of ADH mU/6 hours		Probability a/b
			before dehydration	after dehydration	*a)* before dehydration	*b)* after dehydration	
I.	Non-operated controls	8	2,4±0,441*	6,5±0,236	0,14±0,013	1,99±0,375	$p \ll 0{,}001$
II.	Stalk lesion (diabetes insipidus)	8	22,9±3,507	9,2±1,041	0,06±0,017	0,11±0,038	$p > 0{,}05$
III.	Stalk lesion (regression of diabetes insipidus)	8	8,1±2,297	8,5±0,966	0,07±0,013	0,49±0,097	$p < 0{,}001$
*Standard error							
Probability:	I/II.		$p \ll 0{,}001$	$0{,}02 > p > 0{,}01$	$p < 0{,}001$	$p \ll 0{,}001$	—
	I/III.		$p < 0{,}001$	$0{,}02 > p > 0{,}01$	$p < 0{,}01$	$p < 0{,}001$	—
	II/III.		$p < 0{,}001$	$p > 0{,}05$	$p > 0{,}05$	$p < 0{,}001$	—

of spontaneous water ingestion were influenced by different mechanisms, and the examination of one parameter might have shown a pathological shift when the other yielded normal results. This pointed to the fact that no reliable conclusions concerning water metabolism aught to be drawn on the basis of results obtained by one single method of assay.

The question arose, what factors play a role in the production of the changes in water metabolism following stalk lesion. It could hardly be contested that the diabetes insipidus-like condition developing shortly after operation could be explained by a deficiency of antidiuretic hormone; it was unclear, however, why the characteristic polyuric response observed after water loads disappeared 1 or 2 months later.

The first possibility was that the cessation of the polyuric reaction was correlated with the decrease of the glomerular filtration rate. According to the investigations of BERLINER and DAVIDSON (1956) and KLEEMAN et al. (1957) it is known that in diabetes insipidus the reduction of the glomerular filtration rate moderated polyuria. In previous experiments (KOVÁCS et al. 1959) it was also shown that the polyuria of rats dehydrated by withholding water which suffered from diabetes insipidus ceased completely, furthermore, as a result of an intensive haemoconcentration and the decrease of the renal blood flow the previously polyuric rats hardly excreted any urine.

The examinations have not elucidated the question which mechanisms might evoke the decrease of the glomerular filtration rate in rats which the pituitary stalk had been destroyed in the preceding weeks. From this aspect the hypofunction of the anterior pituitary, that of the ACTH-adrenocortical axis might be primarily involved. Several data suggest that hypophysectomy, adrenalectomy and states associated with hypocorticism resulted in the diminution of the glomerular filtration rate (PICKFORD and RITCHIE, 1945; Boss et al. 1952; BURSTON and GARROD, 1959; ROBERTS and PITTS, 1952; PETERS, 1960). In the rats submitted to stalk lesion the pituitary-adrenocortical system showed hypofunction (MCCANN and BROBECK, 1954; HARRIS, 1955a; FORTIER et al. 1957). This concept was supported by the observations that cortisone administration induced a very pronounced polyuria in rats submitted to stalk lesion some weeks earlier, and that the diabetes insipidus ceased after removal of the adrenals (see below).

The regression of the polyuric reaction can, after all, not be completely explained by the hypofunction of the pituitary-adrenocortical-axis, considering that in the rats with pituitary stalk lesion the function of the adrenal cortex already decreased in the postoperative days (see below), whereas the polyuric reaction could still be demonstrated for some weeks. The other argument concerning the exclusive causative role of the pituitary-adrenocortical-axis was the fact that in some of the rats with stalk lesion after oral tap water loads the diuretic reaction was enhanced even several weeks later, although the function of the pituitary adrenocortical axis decreased. Accordingly the possibility of other mechanisms abolishing the polyuric reaction must be envisaged.

Presumably, a role must be attributed to the fact that the extent of the lesion was smaller in the animals in which the diabetes insipidus regressed; thus the hypothalamo-neurohypophysial system playing a very important

part in the water metabolism might have been only partly damaged. This was also assumed by RANDALL et al. (1960) as already quoted in the introduction. A detailed histological study of the location and extent of the destroyed area, however, did not show any substantial difference between the two groups.

It may be that in the rats submitted to stalk lesion several weeks previously the production of the antidiuretic hormone started again and the cessation of the lack of ADH might be the factor in consequence of which the water metabolism became apparently normal. According to the present investigations the hypothalamo-neurohypophysial system contained visibly more ADH in this regression group. STUTINSKY (1951, 1953; BILLENSTIEN and LEVEQUE (1955) and MOLL (1957, 1958), Kovács (1963) studied serial sections of the hypothalamus and hypophysial stalk from animals hypophysectomized several weeks earlier and found that the stalk cut following surgery was capable of regeneration and a new neurohypophysis-like structure developed in the stump. These morphological observations were supported by the results obtained by LLOYD et al. (1954), MIRSKY et al. (1954) and LLOYD and PIEROG (1955) who found a reappearance of ADH in the blood of animals after hypophysectomy performed several weeks earlier. Our investigations indicated that following stalk lesion the spontaneous urinary ADH secretion was more extensive in the rats in which the enhanced diuretic reaction ceased and in these animals a more considerable adiuretin mobilization could be induced by hyperosmosis than in the persistently polyuric ones. These experiments suggested that the hypothalamo-neurohypophysial system of some of the rats subjected to surgery in the preceding weeks possessed a certain functional capacity which, however, did not reach the level of the non-operated controls.

Our morphological findings did not support this assumption; because signs pointing to a regeneration could not be observed in the stalk, whereas in the posterior pituitary the atrophy was marked. The possibility that the hypothalamus partly assumed the function of the whole system ought also to be taken into account. This was also suggested by RANDALL (1960) and the data that the hypothalamus of such animals contained an almost normal amount of ADH supported this hypothesis as well. The histological studies showed that the supraoptic and paraventricular nuclei were also atrophied in the rats in which the diabetes insipidus had become moderate and that simultaneously Gomori's positive substance also decreased or completely disappeared.

The experiments performed with acethylcholine and ethanol could not either be considered as decisive for the resolution of the problem. Acethylcholine administration caused a short and not intensive antidiuresis in rats a month after stalk lesion. This observation suggested the possibility that the transitory reduction of the urinary output was due to ADH mobilization. This assumption was, however, contradicted by the fact that the same extent of the inhibition of the diuresis could also be seen on the 6—7 th day following stalk lesion when the polyuria in the rats being in a diabetes insipidus-like state was otherwise very pronounced. Thus, it could be assumed that acethylcholine — at least in the applied dose — influenced the urinary output not only by the mobilization of ADH but also by an

other mechanism exerting its effect on renal haemodynamics. The same seems to be valid for the experiments carried out with ethanol. It is known (EGGLETON, 1942, 1949; VAN DYKE and AMES, 1951) that ethanol induced polyuria and that the enhanced diuresis can be attributed to the inhibition of the release of the antidiuretic hormone. The fact that ethanol administration increased the urinary output in animals submitted to stalk lesion some weeks earlier, does not prove the role of ADH, because it may also be supposed that ethanol exerted its effect on the water metabolism by means of some other mechanism.

Chapter V.

THE EFFECT OF PARTIAL LESION OF THE PITUITARY STALK ON WATER METABOLISM IN RATS

1. Histological alterations and changes of the pituitary volume

Recently numerous papers have been published concerning the hormonal and histological alterations developing following lesion of the stalk. The majority of the data, however, deal with the changes occurring after complete lesion of the stalk. Only a few authors (CAMPAGNA et al. 1957; HALÁSZ, 1963; ADAMS et al. 1964; DANIEL et al. 1964) studied the consequences of the partial lesion of the hypophysial stalk.

In the present chapter we describe the results of our experiments including the histological alterations and the changes of the pituitary volumes in rats after partial lesion of the pituitary stalk.

The experiments were carried out on 35 albino rats of both sexes weighing 180—200 g kept on a standard diet. The stalk lesion was per-

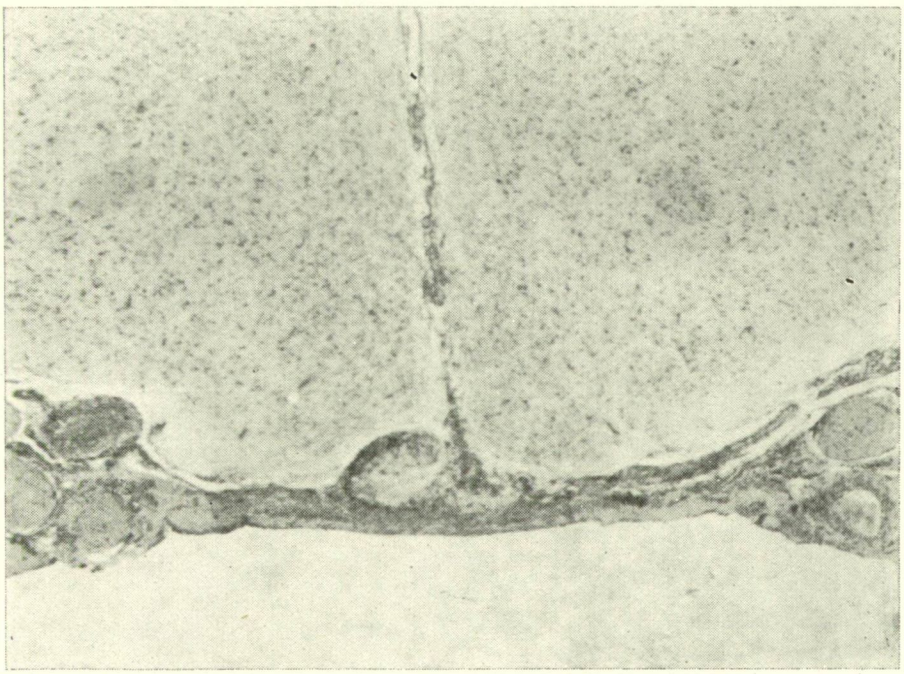


Fig. 29. Typical picture of partial stalk lesion Haem-Eo. staining. 35 X.

formed under nembutal anaesthesia by means of the Horsley—Clarke apparatus. At the end of the experiment the animals were decapitated and autopsied. Histological studies were made of the region of the hypothalamus and pituitary as described in Chapter II. and the location and extent of the damaged area was precisely determined. The typical picture of the total destruction was also describer there. Fig. 29. shows an incomplete stalk lesion. It is well visible that the pituitary stalk located in the lower part of the hypothalamus is only partially destroyed; this type of lesion will corresponding to the literature be termed partial stalk lesion. The animals in which the stalk was hardly impaired, or almost completely destroyed, were omitted at the evaluation.

Volume determinations were only performed in animals in which the histological studies showed a picture of a partial lesion of the stalk. The serial sections mentioned above were used for measuring pituitary volume. The volume of the pituitary expressed in mm^3 was calculated by the method described in Chapter II. which also contains the microphoto of the pituitary of a control rat.

When the histological alterations are analysed 1—2 days after surgery characteristic changes may be found in the pituitary of the rats submitted to lesion of the stalk. One day after the operation characteristic ischaemic necrosis could be observed in the anterior lobe on the side corresponding to the lesion of the stalk, the cell borders were blurred the nuclei showed signs of pyknosis, rhexis and lysis. Two days after the operation homo-

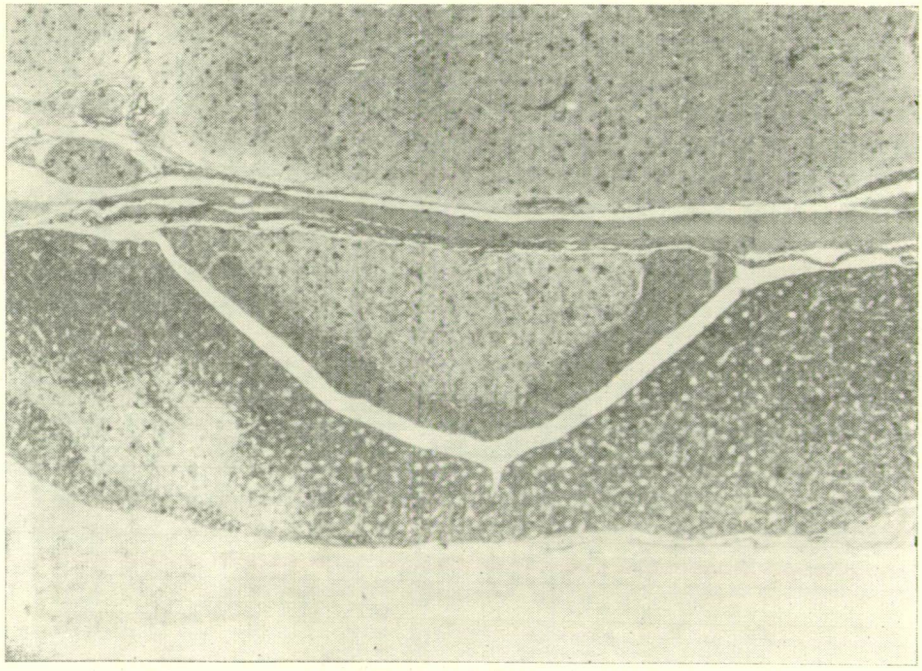


Fig. 30. Pituitary of rat with partial stalk lesion two days after operation. Haem.-Eo. staining. 35 x.

geneous eosinophil necrosis appeared. The smaller dead area was generally located on the right or left of the middle line, only in one part of the anterior lobe and did not extend to the other side. According to histological studies a considerable part of the anterior pituitary remained alive and was composed of intact parenchyma (Fig. 30.).

Observations of some animals (these were not used for volume determinations) showed that 6—8 days after the operation connective tissue formation started in the necrotic areas. Several weeks (4—5 weeks) following stalk lesion necrosis was no more visible, it was replaced by fibrotic scar consisting of connective tissue fibres containing only a few cells (Fig. 31.).

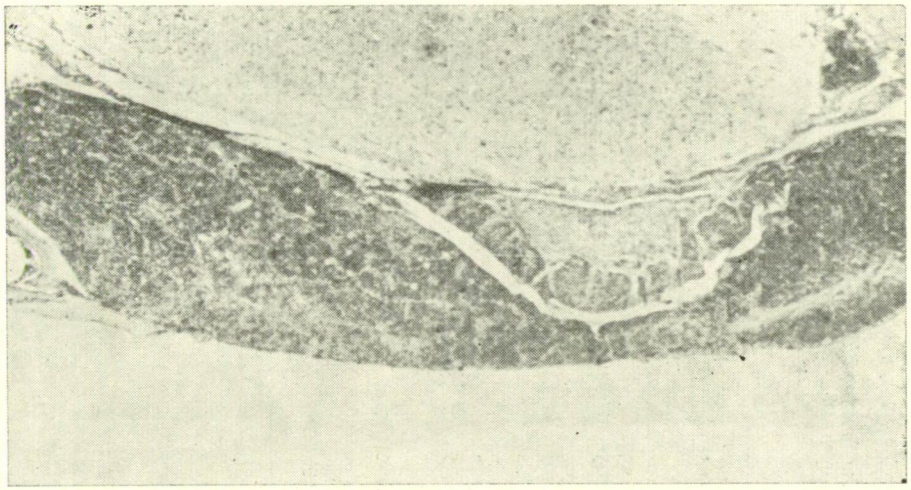


Fig. 31. Pituitary of rat with partial stalk lesion more than 1 mont after operation. Haem.-Eo. staining. 35 x.

In the pars intermedia important changes could not be seen. Immediately after the operation considerable alterations were neither found in the pars nervosa. Later the posterior lobe gradually atrophied and it seemed to contain a larger quantity of cells; the cell nuclei became more dense. Figs. 30. and 31. illustrate the alterations observed.

The quantitative changes are demonstrated in Table 17. It may be seen that in the animals operated some weeks earlier the pituitary volume is moderately reduced. In the anterior pituitary (pars distalis) the necrotic area in the rats submitted to surgery a few days previously was about one fourth of the anterior lobe, later the volume of the destroyed part decreased considerably. An increase of the surviving parenchyma could not be noted in any case. There was no significant change in the volume of the pars intermedia, whereas in the posterior lobe a gradually developing appreciable atrophy could be observed.

Numerous observations suggest that if stalk lesion is performed with different surgical methods not the same alterations develop in the anterior pituitary. Some authors have even noted differences between the same animal species. For instance in monkeys the size of the necrotic area varied (Magoun et al. 1939; Holmes, 1962). Similar results have also been found in

Table 17.

Hypophysial volume in rats with partial stalk lesion

Group		Time elapsed after operation	No of animals	Whole pituitary mm³	Pars distalis			Pars intermedia mm³	Pars nervosa mm³
					whole mm³	destroyed portion mm³	intact portion mm³		
I.	Intact	—	10	6,688 ±0,303*	5,020 ±0,209	—	5,020 ±0,209	0,474 ±0,027	1,194 ±0,112
II.	Pituitary Stalk lesion	1—2 days	10	6,010 ±0,201	4,747 ±0,277	1,309 ±0,084	3,467 ±0,246	0,475 ±0,039	0,789 ±0,071
III.	Pituitary Stalk lesion	More than a month	15	4,055 ±0,275	3,302 ±0,260	0,318 ±0,026	2,988 ±0,265	0,455 ±0,011	0,297 ±0,027
* Standard error			I/II.	$p>0,05$	$p>0,05$	—	$p<0,001$	$p>0,05$	$p<0,001$
		Probability:	I/III.	$p<0,001$	$p<0,001$	—	$p<0,001$	$p>0,05$	$p<0,001$

human material (RUSSELL, 1956; LUNDBAEK et al. 1960). In experiments carried out in goats DANIEL and PRICHARD (1958) observed that in the majority of the animals the anterior pituitary was almost completely destroyed, however, in one of their cases the necrosis was only small. In one fourth of rats with stalk lesion ADAMS et al. (1962) did not find widespread necrosis. According to our present investigations the fact whether the stalk lesion was complete or not affords an explanation for the differences of the results. RUSSELL (1956) studying human autopsy material drew similar conclusions.

ADAMS et al. (1963) observed that after complete lesion of the stalk extensive symmetrical necrosis develops in rats. Following electrolytic lesions DANIEL and PRICHARD (1956) and ADAMS et al. (1964) found in cases in which the lesion was not located in the middle line small necroses localized on one side of the anterior lobe. On examining the pituitary volume of the partially stalk lesioned animals it may be established that the necrosis in the anterior pituitary is smaller than after complete lesion, this is in the first place due to the fact that only a part of the portal vessels is damaged. The necrosis may always be found on the side corresponding to the lesion. Thus it may be suggested that the blood supply of the anterior pituitary has a segmental character meaning that among the vessels supplying the single areas there are no or only very few collateral anastomoses. ADAMS et al. (1964) and DANIEL et al. (1964) reached the same conclusions.

Concerning the pars intermedia many contradictory data can be found in the literature. Some of the authors (UOTILA, 1939; BARRNETT and GREEP, 1952; DANIEL and PRICHARD, 1958; HÁMORI, 1960; HOLMES, 1962) reported an increase of the volume, others did not observe a change (DANIEL and PRICHARD, 1956; CAMPBELL and HARRIS, 1957; HOLMES, 1961; ADAMS et al. 1963). Our investigations described in the previous chapters indicate that following complete lesion of the stalk hypertrophy of the pars intermedia did not ensue. Similar results were also obtained in the rats with partial stalk lesion.

In chapter II. in accordance with the unequivocal observations of other authors (MAGOUN et al. 1939; BARRNETT and GREEP, 1951; DANIEL and PRICHARD, 1956, 1958; CAMPBELL and HARRIS, 1957; HOLMES, 1961; 1962; ADAMS et al. 1963) we also described a pronounced atrophy of the posterior pituitary. It seems worth while to mention that we also found considerable atrophy in the neural lobe of the partially stalk lesioned rats. The correlations between this phenomenon and the functional state of the posterior lobe are still unknown; to elucidate this question further experiments are needed.

2. *Examinations of the water metabolism in the period following partial destruction of the pituitary stalk*

On analysing the changes in the water metabolism following complete lesion of the stalk it was striking that certain alterations could be found even after partial stalk lesion. Therefore, it seemed worth while to study in detail the water metabolism of rats with partial pituitary stalk lesion. Observations made in this connection will be reported in this chapter.

Eighty-four albino rats of either sex, weighing 180—200 g maintained on a standard diet were used. The pituitary stalk was destroyed under nembutal anaesthesia by means of a Horsley—Clarke apparatus. At the end of the experiment after killing the animals the region of the hypothalamus was controlled by histological studies as described in the previous chapter. Fig. 29. demonstrates a typical picture of a partial stalk lesion. The animals in which the stalk was damaged only slightly or destroyed completely, were excluded from the evaluation. Six rats with unilateral lateral focal hypothalamic lesions in the premamillary region and further six animals with focal subcortical lesions in the parietal area served as controls. In these animals the pituitary stalk was left intact.

The changes in the water metabolism were studied daily. Ten rats were kept in individual cages and their 24-hour water intake was measured before and after the operation.

In another group the diuretic reaction to oral water loading was studied. The animals were fasted for 10 hours before the experiment. Tap water was allowed ad libitum (except for the water deprived group) and, in addition, every animal received ten ml of tap water daily through a stomach tube for 1 week before the planned day of operation in order to avoid dehydration.

Water loading was carried out as described previously. The results were expressed by a numerical value characterising the diuresis curve of the animals as summation urine output. One group (8 rats) received instead of tap water 5 per cent/body weight of physiological NaCl solution and another group (8 rats) 5 per cent ethanol. Eight rats with partial stalk lesions were treated with ten mg of cortisone subcutaneously for 1 week

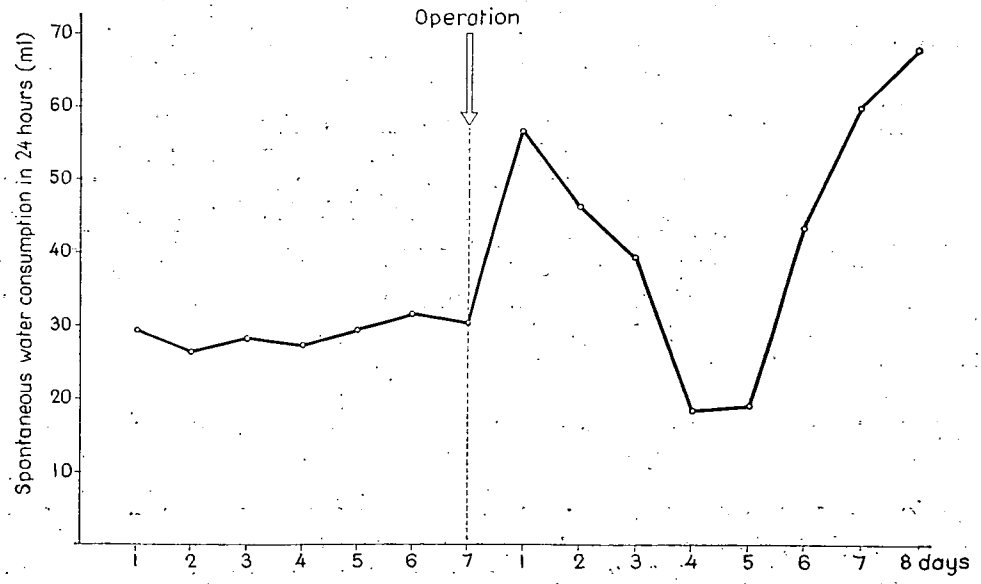


Fig. 32. Daily spontaneous water consumption following partial destruction of the pituitary stalk

after the operation. For comparison, we also studied the water metabolism of the non-operated control rats. Another group of rats was fed a dry diet for 8 days and no drinking water was allowed. Eight of the water deprived rats were not operated; this was the control group. The other 8 dehydrated

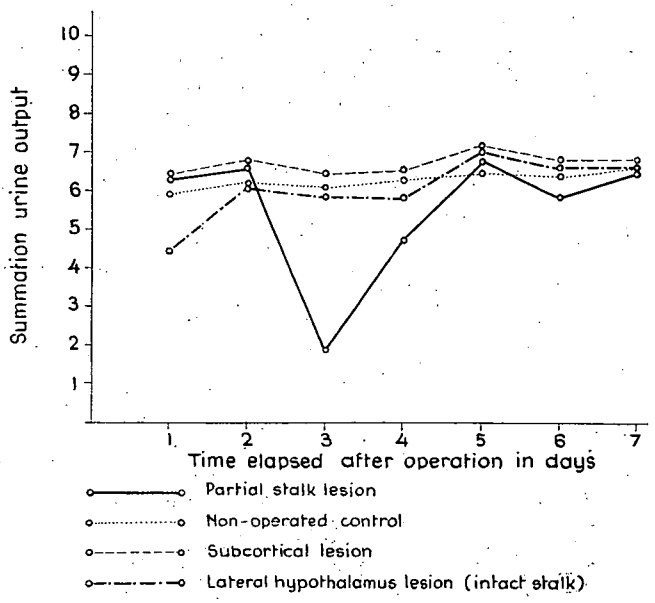


Fig. 33. Summation urine output of intact rats and of rats with partial stalk, lateral hypothalamus or subcortical lesions following orally administration of tap water

rats were subjected to partial stalk lesion. After operation this group, too, was allowed to drink tap water ad libitum.

The results are presented in Figures.

Fig. 32. shows the average spontaneous 24-hour water intake of 10 rats. The results obtained in the pre-operative period were used as controls. As can be seen before the operation the daily water intake varied from 25—31 ml, with no major fluctuation on the single experimental days. Following partial destruction of the stalk, water consumption rose, then decreased below the initial value, then increased again.

It seemed necessary to determine whether in the period of reduced water intake the animals were oliguric after water loading, and whether this phenomenon was connected with the stalk lesion. Therefore, the water metabolism of the rats with partial stalk lesion was compared with that of the non-operated controls, further with that of animals bearing lesions not affecting the stalk (subcortical, lateral hypothalamic). Fig. 33. shows that the oliguric phase had developed in the rats with partial stalk lesion, while no noteworthy change occured during the 7 days of observation in the non-operated controls and the animals with lesions not affecting the stalk. In the same period the animals with partial stalk lesion subjected to oral water loading showed no polyuria.

Next, it was attempted to prevent the development of the oliguric phase. Eight stalk lesioned rats subjected to water loading were treated with cortisone; further ten rats were subjected to oral loading with 5 per cent ethanol and eight with physiological saline, instead of tap water. Fig. 34. shows that diuresis decreased in each of the three groups. However, the figures shown indicate that the oliguria was milder. It should, however, be remarked that these average values are not quite reliable. Antidiuresis developed in every rat with a stalk lesion, but as in some animals the water retention appeared on the third, and in others on the fourth day, the unselected calculated values are higher than the real ones. The diuretic reaction of the animals treated with cortisone or ethanol was slightly more marked, while that of those treated with saline less marked, than the

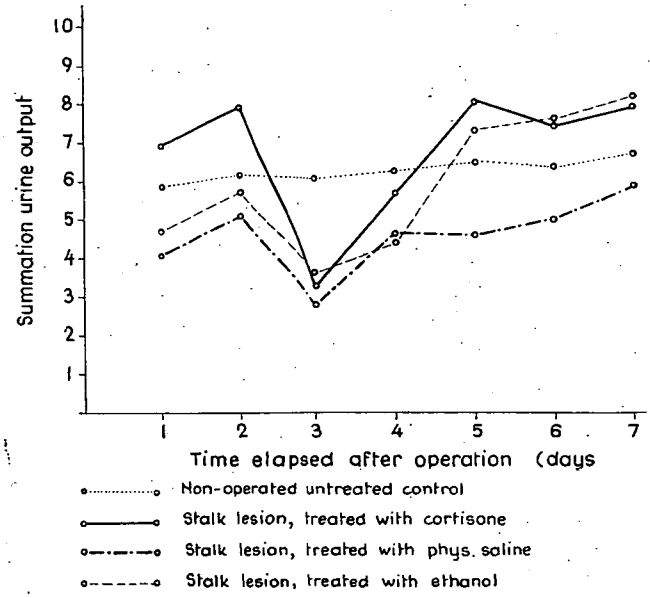


Fig. 34. Effect of cortisone, 0,9% NaCl solution and ethanol on summation urine output of rats with partial stalk lesion

average. The same differences were observed in the corresponding non-operated control groups.

Fig. 35. shows the changes of water metabolism of the rats deprived of water before partial destruction of the stalk. It can be seen that the water-deprived, non-operated group excreted less urine on the first day; in the subsequent days, however, there was no notable difference between the non-operated hydrated and the water-deprived animals. In the hydrated rats with lesions of the stalk antidiuresis was transitory, while in water deprived animals there was no oliguric interphase and the urinary volume increased gradually.

In previous chapters the disturbances in water metabolism following total destruction of the pituitary stalk were analysed. The experiments described in this chapter indicate that an oliguric interphase appears also

after partial destruction of the stalk. Similar results were obtained in the dog by CAMPAGNA et al. (1957). The antidiuretic period is connected with the stalk lesion, as it is absent in animals with an intact stalk bearing subcortical or lateral hypothalamic lesions.

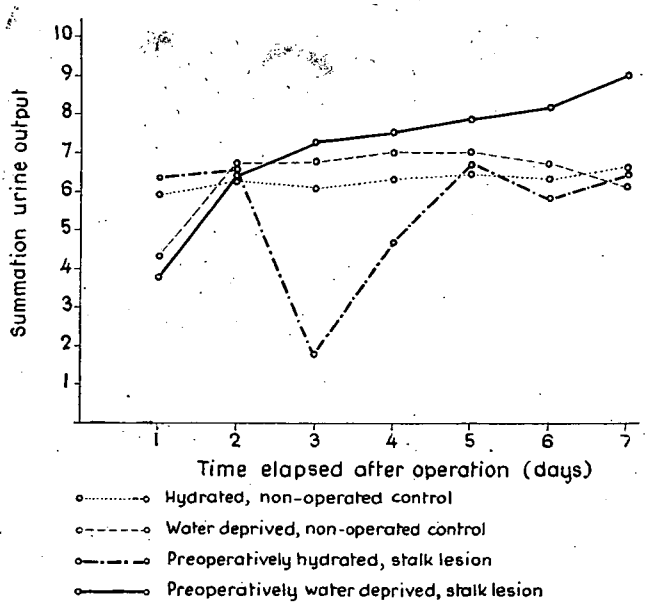


Fig. 35. Effect of preoperative water deprivation on the summation urine output of intact rats and of rats with partial stalk lesion

LIPSETT and PEARSON (1957) assumed that lack of cortisone may play a role in the induction of the interphase. This interpretation is not supported by our experiments, as our results prove that in the development of the oliguric phase the hypofunction of the hypophysial-adrenocortical axis resulting from the stalk lesion does not play a role, because the administration of cortisone did not abolish the interphase. Similar results were obtained when the animals were treated with saline or ethanol.

The investigations described in Chapter III. have suggested that the oliguric phase was due to an increased output of antidiuretic hormone. The present results — (the interphase could also be observed following partial lesion of the stalk, for its development the stalk must be injured, in this period the water intake of the animals decreased, too) — are is harmony with our earlier findings, and are further supported by the fact that the phenomenon failed to appear in rats deprived of water before the operation. Prolonged water deprivation is namely known to cause ADH depletion (SIMON, 1934; SIMON and KARDOS, 1934; ORTMANN, 1951; ZETLER, 1952; EICHNER, 1953; KOVÁCS et al. 1954; KOVÁCS, 1957).

3. *Investigation of water metabolism some weeks after partial destruction of the pituitary stalk*

In the first part of this chapter the histological alterations and the changes in the volume of the pituitary were described after partial destruction of the pituitary stalk. It was established that immediately after the operation a focal necrosis could be observed in the anterior pituitary; later in this areas fibrosis, atrophy appeared and the volume of the anterior pituitary decreased. Following the manipulation a significant atrophy developing gradually in the neural lobe was seen. The observation that after partial destruction of the stalk the oliguric interphase also developed in these animals, pointed to the injury of the posterior pituitary.

Therefore it seemed advisable to study the changes in the water metabolism of rats which had been submitted to partial stalk lesion some weeks previously.

This subchapter describes the results of these experiments.

The experiments were performed on albino rats of both sexes weighing 170—220 g kept on a standard diet. The lesion of the stalk was carried out under nembutal anaesthesia by means of a Horsley—Clarke apparatus. At the end of the experiment the animals were killed by decapitation and autopsied. The histological studies and the typical picture of the partial stalk lesion have been described in detail previously. The animals in which the stalk was damaged only slightly, or destroyed completely, were excluded from the evaluation.

The changes in the water metabolism of the animals were recorded by applying different methods. One group of the animals was placed in single cages and their spontaneous water intake during 24 hours was measured.

In the other groups of the rats the diuretic reaction developing after oral water loads was investigated (for description of the method see above). To some rats 10 mg cortisone was administered every day subcutaneously during four days prior to the examination (Adreson, Organon, Oss). Another group received 5 per cent/body weight of physiological NaCl solution or 5 per cent ethanol instaed of tap water.

Subsequently the sodium, potassium, chloride and creatinine excretion of the rats was also determined after oral administration of tap water and physiological NaCl solution in amounts of 5 per cent/body weight. The experiment lasted for 5 hours. At the beginning of the experiment the animals were given orally 5 per cent/body weight of tap water and physiological NaCl by a stomach tube. At the end of the fifth hour the quantity of the urine was measured with an accuracy of 0,1 ml and then the sodium, potassium, chloride and creatinine concentration of the urine samples was estimated. The sodium and potassium determination was performed by means of a flame photometer, that of chloride by the procedure of SCHALES and SCHALES (1941) and that of creatinine by the method of FOLIN—WU (1919) modified according to BROD—SIROTA (1948). The quantity of the urinary output was computed for 100 g body weight. The sodium, potassium and chloride values were expressed in concentration (mEqu/lit); the total amounts expressed in μ/equiv. computed for 100 g body weight excreted within 5 hours were also presented. The creatine values are expressed in mg/100 g body weight.

The specific gravity of the urine was determined by the method of GÁL et al. (1953) using a pyknometer.

The antidiuretic hormone estimations were performed on female rats under alcohol (ethanol) anaesthesia by the method of DE WIED (1960). The method with which the urine samples were prepared and the extractions from the posterior pituitary and hypothalamus were made have already been described above.

The results were biometrically analysed using Student's „t" test.

Table 18.

Daily spontaneous water consumption of intact rats and of rats with partial stalk lesion operated more than a month previously

Group		No. of animals	Body weight g	Spontaneous water consumption 24 h/ml	Probability
I.	Inract	30	216,0 ±5,8*	25,5 ±1,1	$p>0,001$
II.	Partial Stalk lesion	30	185,7 ±3,5	49,2 ±5,5	

* Standard error

Table 18. shows the average values of the spontaneous water intake during 24 hours of rats submitted to partial stalk lesion a month previously. It could be established that the operated animals consumed more water than the non-operated controls. This difference is still more pronounced considering that the body weight of the rats with stalk lesion was less than that of the controls.

A study of the diuretic reaction suggested that following oral water loads the urinary output of rats with pituitary stalk lesion did not increase. These observations can be seen in Table 19. It is well visible that oral administration of cortisone induced a mild, and that of physiological NaCl solution a significant, polyuric reaction. On applying 5 per cent ethanol loads instead of tap water the urinary excretion of the non-operated rats increased. Following ethanol treatment the diuretic reaction of the animals with lesions of the stalk also rose, however, these values did not reach the average of those of the controls.

Table 20. represents the results of the electrolyte- and creatinine output. It could be noted that the average urinary output of the orally water loaded animals submitted in the preceding weeks to partial lesion of the stalk did not differ substantially from that of the non-operated controls. The sodium and potassium concentration of the urine and their excretion during 5 hours was considerably less as compared to that of the controls. As to the chloride excretion there was no appreciable difference between the groups. In this experimental series after oral administration

Table 19.

Effect of cortisone, phys. NaCl, or ethanol on summation urine output of intact rats and rats with partial stalk lesion operated more than a month previously

Group		Orally administered fluid	Treatment	No. of animals	Body weight g	Summation urine output	Probability	
I.	Intact	Tap water	—	30	179,8 ±5,3*	6,61 ±0,20	I/II.	$p>0,05$
II.	Intact	Tap water	cortisone	30	181,0 ±3,0	6,84 ±0,16	I/III.	$p\ll0,001$
III.	Intact	Phys. NaCl	—	30	226,3 ±6,0	3,87 ±0,26	I/IV.	$p<0,001$
IV.	Intact	Ethanol	—	24	172,5 ±3,0	8,27 ±0,16	I/V.	$0,02>p>0,01$
V.	Partial stalk lesion	Tap water	—	24	186,9 ±3,5	5,93 ±0,19	II/VI.	$p<0,001$
VI.	Partial stalk lesion	Tap water	cortisone	20	173,2 ±2,6	7,94 ±0,22	III/VII.	$p<0,001$
VII.	Partial stalk lesion	Phys. NaCl	—	30	181,0 ±3,4	6,39 ±0,37	IV/VIII.	$p<0,001$
VIII.	Partial stalk lesion	Ethanol	—	17	170,6 ±3,1	7,19 ±0,22	V/VI.	$p<0,001$
							V/VII.	$p>0,05$
							V/VIII.	$p<0,001$

*Standard error

Table 20.

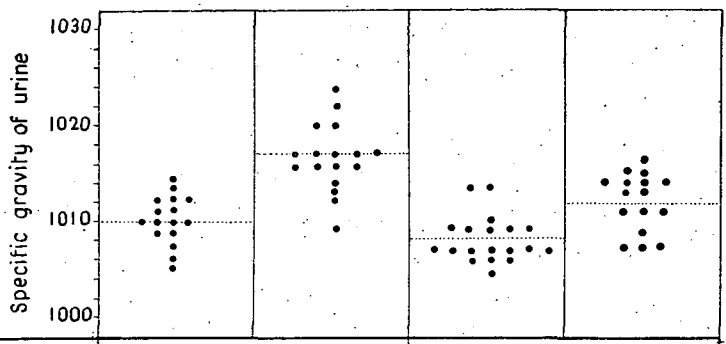
Sodium, potassium, chloride and creatinine excretion of intact rats and of rats with partial pituitary stalk lesion more than 1 month after the operation following the oral administration of tap water or phys. NaCl

Group		Fluid asministered	No. of animals	Body weight g	Amount of urine excereted in 5 h calculated for 100 g boody weight ml	Amount of creatinine excreted in 5 h calculated for 5h calculated 100 g body weight mg/100 g body weihgt	Concentration (m Eq/l)			Amount excreted in 5 hours calculated for 100 g body weight (μEq) 100 g body weight		
							Na	K	Cl	Na	K	Cl
I.	Intact	Tap water	74	181,9 ±6,1*	5,0 ±0,2	0,48 ±0,03	31,0 ±2,5	15,8 ±1,8	38,9 ±2.9	161,1 ±12,9	79,8 ±9,9	196,0 ±17,6
II.	Partial Stalk lesion	Tap water	18	181,4 ±3,9	4,2 ±0,3	0,41 ±0,04	21,4 ±2,4	10,3 ±1,1	44,1 ±10,1	91,0 ±13,9	42,8 ±7,0	149,6 ±28,7
III.	Intact	Phys. NaCl	16	185,9 ±4,5	4,2 ±0,3	0,54 ±0,05	140,0 ±5,5	27,8 ±1,8	182,3 ±4,9	584,3 ±53,6	117,6 ±14,3	754,9 ±54,4
IV.	Partial Stalk lesion	Phys. NaCl	20	178,5 ±3,9	4,9 ±0,4	0,65 ±0,06	146,6 ±12,0	24,5 ±2,3	160,6 ±12,6	691,7 ±40,2	111,9 ±9,6	716,3 ±38,8
Probability:					I/II.	0,05>p>0,02	p>0,05	p<0,01	0,20>p>0,01	p>0,05	p<0,001	p<0,01	p>0,05
					I/III.	0,05>p>0,02	p>0,05	$p\ll$0,001	p<0,001	$p\ll$0,001	p<0,001	0,05>p>0,02	$p\ll$0,001
					II/IV.	p>0,05	p>0,01	$p\ll$0,001	p<0,001	p<0,001	$p\ll$0,001	p<0,001	$p\ll$0,001
					III/IV.	p>0,05	p>0,05	p<0,05	p>0,05	p>0,05	p>0,05	p>0,05	p>0,05

* Standard error

of physiological saline the urinary output of both the operated and control animals did not show any substantial difference from the values of the animals subjected to water loads. Following administration of physiological NaCl the creatinine excretion rose slightly; in the operated animals it was more pronounced. A comparison of the values of the rats loaded with tap water with those of the animals loaded with physiological saline showed that after administration of physiological NaCl not only more Na and Cl, but more K was excreted, as well.

Between the electrolyte excretion of the operated and the non-operated animals there was no considerable difference.



Group	Intact		Partial stalk lesion	
Fluid administered orally	Tap water	phys. NaCl	Tap water	phys. NaCl
Number of animals	16	18	20	16
Body weight g	198·1 ± 3·2 *	194·4 ± 4·1	186·5 ± 2·9	181·0 ± 3·8
Specific gravity of urine	1010 ± 0·62	1017 ± 0·83	1008 ± 0·48	1012 ± 0·76

* Standard error

Fig. 36. Specific gravity of urine of intact rats and of rats witz partial pituitary stalk lesion operated more than 1 month previously after oral administration of tap water and phys. NaCl.

Fig. 36. illustrates the specific gravity of the urine excreted within 5 hours of the different groups. It may be seen that the specific gravity of the urine proved to be somewhat lower in rats with partial stalk lesion after physiological saline loads as compared to the non-operated controls. After water loads there was no appreciable difference between the specific gravity of the urine of the intact group and the group of animals bearing partial stalk lesions.

The urinary ADH excretion and the ADH content of the posterior lobe and the hypothalamus is presented on Table 21. The Table shows that the operated rats did not excrete significantly less ADH. On the other hand, a decrease in weight of the neurohypophysis could be demonstrated in the animals with partial lesion of the stalk. The antidiuretic hormone content of the posterior lobe decreased, as well, whereas in the hypothalamus only slightly less hormone could be found as compared to the values of the controls.

Table 21.

ADH secretion, weight of neurohypophysis, ADH content of hypothalamoneurohypophysial system of control rats and rats with partial pituitary stalk lesion operated more than 1 month previously

	Group	No. of animals	Body weight g	ADH secretion mU/24 h	Weight of neuro-hypophysis mg	ADH content of neuro-hypophysis mU	ADH content of hypothalamus mU
I.	Control	8	201,0±6,7*	0,43±0,050	0,94±0,048	1118,4±47,5	141,1±12,8
II.	Partial Stalk lesion	8	217,4±8,3	0,38±0,063	0,53±0,039	483,7±21,2	70,5±6,8
* Standard error			Probability:	$p>0,05$	$p<0,001$	$p\ll0,001$	$0,02>p>0,01$

Our investigations indicated that several weeks after the partial destruction of the stalk the spontaneous water consumption of the rats during 24 hours was enhanced. On the other hand, after water loads polyuria did not develop in the animals bearing partial lesions of the stalk. The results obtained with examinations by means of the two adequate methods used to study the water metabolism did not show in this case similar changes. In the experiments dealing with the water metabolism of animals with total destruction of the pituitary stalk the same conclusion could be drawn. Our investigations suggest that for the demonstration of the moderate changes in the water metabolism following partial lesion of the stalk the measurement of the spontaneous water intake during 24 hours is more suitable.

It was demonstrated that also in rats with partial stalk lesion the oliguric interphase associated with enhanced ADH release developed. Some weeks after the operation the marked decrease of the volume of the posterior pituitary became striking and a disturbance of the water metabolism was found in the animals. (The water intake was enhanced, following water loads the electrolyte excretion decreased.) Our investigations suggest that these divergences are in correlation with the ADH system. The posterior pituitary and the hypothalamus contained less antidiuretic hormone after partial descruction of the pituitary stalk. The above assumption is also supported by the fact that after saline loads the diuretic reaction of animals with partial destruction of the stalk performed some weeks earlier was more pronounced. It is known that this phenomenon can be observed in a state associated with diminished ADH secretion (FRIEDMAN et al. 1958; ALEXANDER, 1959).

The impairment of the adiuretin system can from the functional aspect, however, only be considered as partial. The moderate disturbance of the water metabolism could only be disclosed after the application of some special methods (water intake, saline loads). On the other hand, the observations, that the diuretic reaction developing after water loads remained normal during the whole postoperative period and that the ADH secretion did not differ substantially from the control values, suggest that the functional capacity of the ADH system in rats bearing partial lesions of the stalk is not markedly decreased and is almost normal.

Chapter VI.

FUNCTION OF THE ACTH-ADRENOCORTICAL AXIS IN RATS WITS PITUITARY STALK LESION

1. The role of the adrenal cortex in the water metabolism of rats with stalk lesion

It is known for a long time that in the regulation of the water metabolism both the adiuretin-system and the ACTH-adrenocortical axis play an important role. SILVETTE and BRITTON had reached as far back as 1938 the conclusion that there is an antagonism between the two systems. Diabetes insipidus occurring when the tissues producing ADH are demaged does not develop if simultaneously hypofunction of the adrenal cortex is present. Numerous clinical observations suggest (SKILLER et al. 1956; RIBANDO, 1958; REES and ZILVA, 1959) that in Addison disease polyuria does not occur if the lesion of the posterior pituitary ensues, as well. In animal experiments (GAUNT et al. 1949; GAUNT, 1951; CHESTER JONES, 1957; PENTZ, 1957; PENTZ et al. 1957; SMITH and TYREE, 1957; KENNEDY and CRAWFORD, 1961) an increase in the urinary output could not be observed following neurohypophysectomy or after total body X-ray irradiation if the adrenals had been previously removed. According to clinical and experimental data (v. HANN, 1918; INGRAM and WINTER, 1938; BIGGART and ALEXANDER, 1939; DINGMAN et al. 1958; REES and ZILVA, 1959) polyuria cannot either be observed following injury of the posterior lobe in the case of hypofunction or removal of the anterior pituitary. On the other hand, a change in the function of the adrenal cortex also influences the intact ADH system. Many authors (MARTIN et al. 1939; BIRNIE et al. 1949; KOVÁCS and BACHRACH, 1951; LEAF and MAMBY, 1952; LLOYD, 1952; CAVALLERO et al. 1954; GINSBURG, 1954; MIRSKY et al. 1954a, b) reached the conclusion that after adrenalectomy the amount of the antidiuretic hormone increases in the blood and decreases in the posterior pituitary and in detail how adrenalectomy influences the characteristic changes in the developing after the destruction of the adrenal cortex to this mechanism.

The data of the literature, however, fail to agree on these questions. The decision is made difficult by the circumstance that the experiments performed on different animal species and by various methods often lead to contradictory results. The purpose of this part of our work is to analyse in detail how adrenalestomy influences the characteristic changes in the water metabolism following pituitary stalk lesion in rats.

Albino rats of either sex, weighing 180—220 g maintained on a standard diet were used for the experiments. The pituitary stalk lesion was performed under pentobarbital anasthesia, by means of a Horsley—Clarke apparatus. On completion of the experiment the animals were killed by decapitation and autopsied. The histological studies and the typical

picture of the destruction have been described in detail in Chapter II. The animals in which according to the histological examinations the stalk lesion was not complete were excluded at the evaluation of the results. The adrenalectomy was performed under ether anaesthesia by the usual lumbal approach. At the end of the experiment when the animals were autopsied the site of the adrenals was carefully examined. The animals in which the adrenalectomy was not complete were also discarded from the evaluation. To one group of the animals cortisone (Adreson, Organon, Oss) was also administered for 7 days daily in subcutaneous doses of 10 mg/rat.

The water metabolism of the rats was recorded daily in the postoperative period. The rats kept in individual cages were given orally by a stomach tube 5 per cent/body weight of tap water. The other group of the animals got instead of tap water 0,9 per cent or 2 per cent NaCl solution ad libitum and the same solutions were used for the examinations with loads. The excreted urine was measured in intervals of one hour for 8 hours with an accuracy of 0,1 ml. The results were expressed as summation urine output by a number characterising the diuresis curve of the animals. The detailed description of the method can be found in Chapter III.

It was also described there that in rats after destruction of the pituitary stalk a characteristic polyphasic disturbance of the water metabolism developed: the postoperative polyuria lasting for 1—2 days was followed by a temporary oliguric interphase; subsequently a more persistent polyuria could be observed. The observations will not be reported in detail here they will be only mentioned for the sake of the correct evaluation of the results.

In the first part of the examinations the question was dealt with how adrenalectomy following stalk lesion influenced the changes of the water metabolism described above in the case of water loads. The adrenals were removed on the fourth postoperative day. This period was chosen for several reasons: first of all it was only possible to gain information in this way whether or not following destruction of the stalk the diuresis increased in the experimental animals. This could be seen in the transient polyuric period (in the 1—2 postoperative days) and conclusions could be drawn whether the development of typical diabetes insipidus could be expected. If the two operations had been performed simultaneously a decision could not have been reached whether the inhibition of the diuresis was the result of the oliguric interphase following destruction of the stalk, or that of adrenalectomy.

The results are presented in Fig. 37. It may be seen from the Fig. that the summation values (the mean value of 10 animals) of the non-operated control rats varied between 5,5 and 7,0 and did not show a considerable fluctuation on any of the experimental days. The urinary output of the adrenalectomized animals (10 rats) showed a gradual reduction; in the first 4 days after the adrenalectomy a substantial diminution as compared to that of the controls could not yet be observed, however, from the fifth day on an inhibition of the diuresis could already be established. The rats with stalk lesions were divided into 2 groups: the first group (10

animals) was not submitted to adrenalectomy and the characteristic triphasic curve was obtained. The second group (9 animals) was adrenalectomized on the fourth day following destruction of the stalk at the maximum of the interphase. The Fig. shows that in these rats the urinary output also increased, but it was not so pronounced as in the animals with

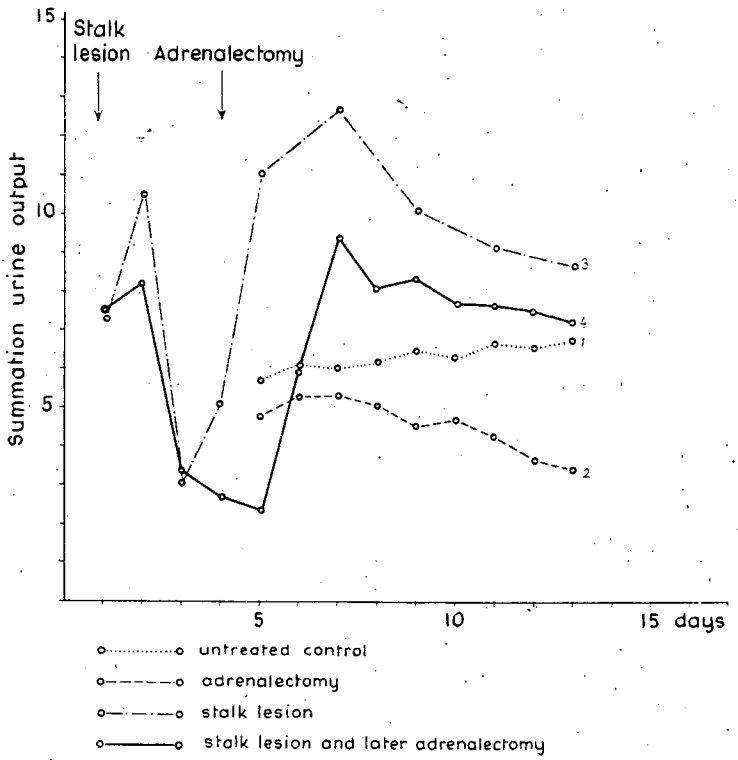


Fig. 37. Summation urine output of rats with stalk lesion and adrenalectomy after tap water load

lesions. On the other hand, the polyuric reaction definitely showed a decreasing tendency and on the 5—6 th day after the adrenalectomy it approached the values of the non-operated controls. It should also be mentioned that the animals operated twice tolerated the operation very badly and two thirds of these rats died a few days after the adrenalectomy. The Fig. presents only the urinary output of the animals in a good state.

Subsequently it was attempted to elucidate the question if in previously adrenalectomized rats an increase of the urinary output developed following stalk lesion. The results may be seen in Fig. 38. It is visible that in the animals only submitted to adrenalectomy (10 rats) oliguria ensued gradually. Whereas in the rats (10 rats) in which only a lesion of the stalk had been performed, the typical triphasic curve could be found. The adrenalectomy was carried out 5 days before the stalk lesion as

according to the previous Fig. this period is necessary for the development of a more considerable inhibition of the diuresis. The Fig. demonstrates that rats submitted earlier to adrenalectomy and then to stalk lesion (8 rats) did not show a polyuric reaction. Whilst after cortisone administration applied daily, after the fifth day of the destruction the urinary output increased considerably, an intensive polyuria developed in these rats.

In the following the observations of CAVALLERO et al. (1954) were taken into consideration who found that the urinary excretion of adrenalectomized rats became normal following NaCl administration. Other authors (CHESTER JONES, 1957; FRIEDMAN et al. 1958; ALEXANDER, 1959) on the other hand, observed that the diabetes insipidus increased after saline loads. It seemed necessary to examine the effect of saline on the urinary output of non-operated or adrenalectomized rats. The results are illustrated in Fig. 39. Following tap water loads (Fig. 39/a) the summation urine output of the animals varied between 5,26 and 6,29 and did not show considerable fluctuations any days of the experiment. Conversely, if the adrenals were removed previously the amount of the urine gradually diminished. To the other group of animals physiological saline was administered (Fig. 39/b). It may be seen that the diuretic reaction of the non-operated rats also corresponded in this case to the normal level and was

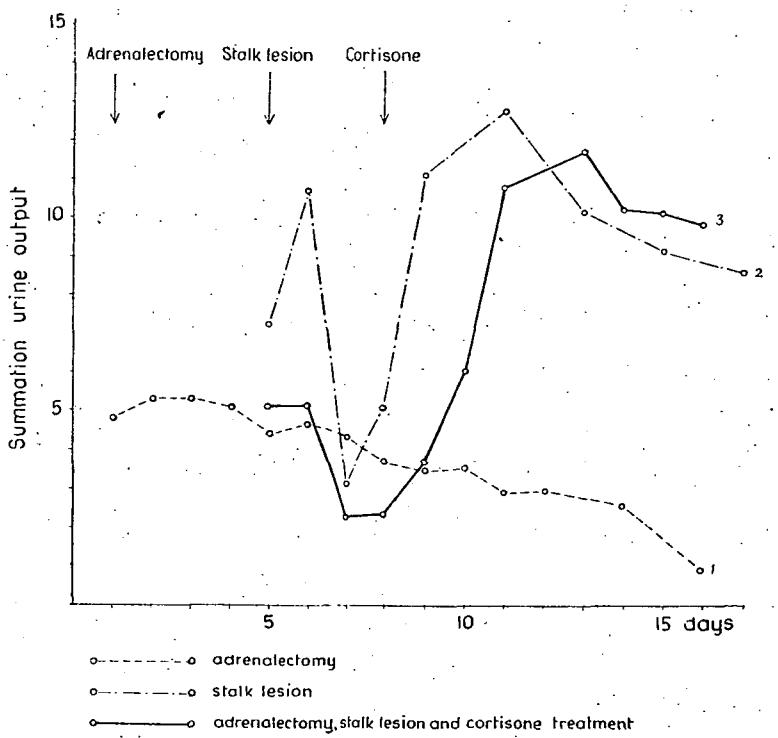


Fig. 38. Summation urine output of rats with adrenalectomy and stalk lesion after tap water load

even slightly more marked than of the animals submitted to tap water loads. On the other hand, after administration of 0,9 per cent NaCl solution the urinary output of adrenalectomized animals did not reach the average values of the non-operated controls. As contrasted to the water

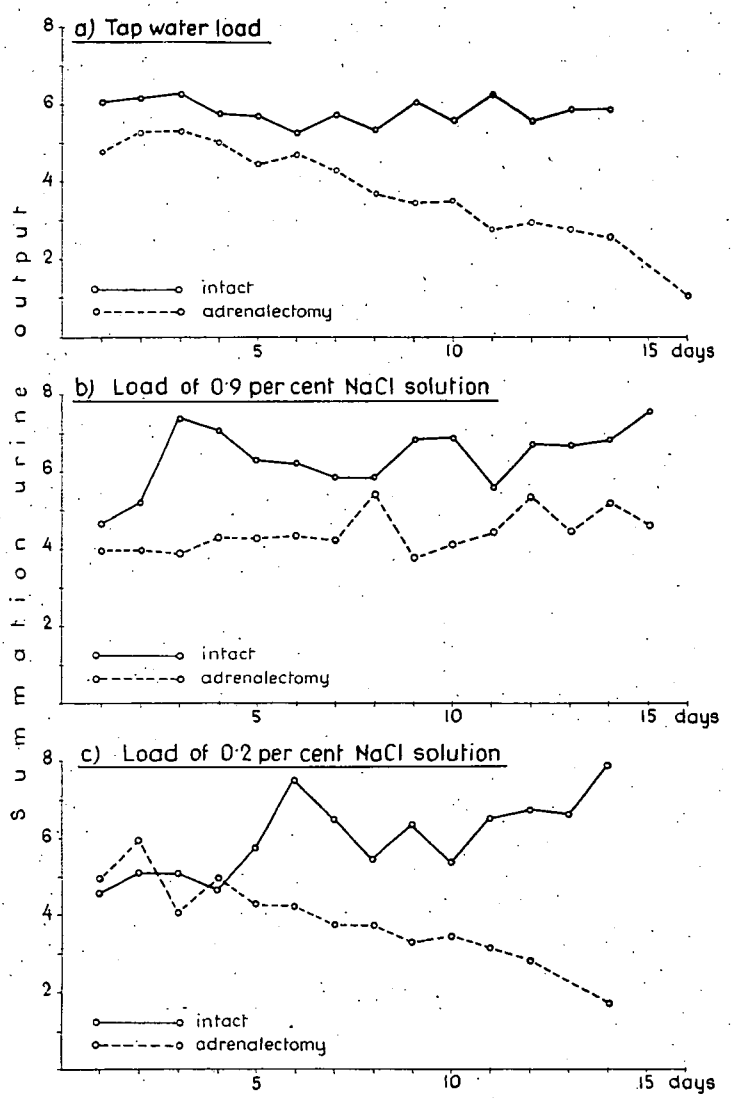


Fig. 39. Summation urine output of intact and adrenalectomized rats after the oral administration of a solution of 0,9 per cent or 2 per cent NaCl

loaded, adrenalectomized rats, the only deviation was that the state of the operated animals who had received a physiological saline solution was far better and in the second phase of the experiment only a slight, but not significant inhibition of the diuresis ensued. Also after administration

of a 2 per cent NaCl solution (Fig. 39/c) a normal diuretic reaction could be observed in the non-operated animals. Later, however, the state of the animals gradually deteriorated. The diuresis curve of the adrenalectomized rats substantially agreed with the values of the operated animals submitted to tap water loads. Each group contained 10 rats.

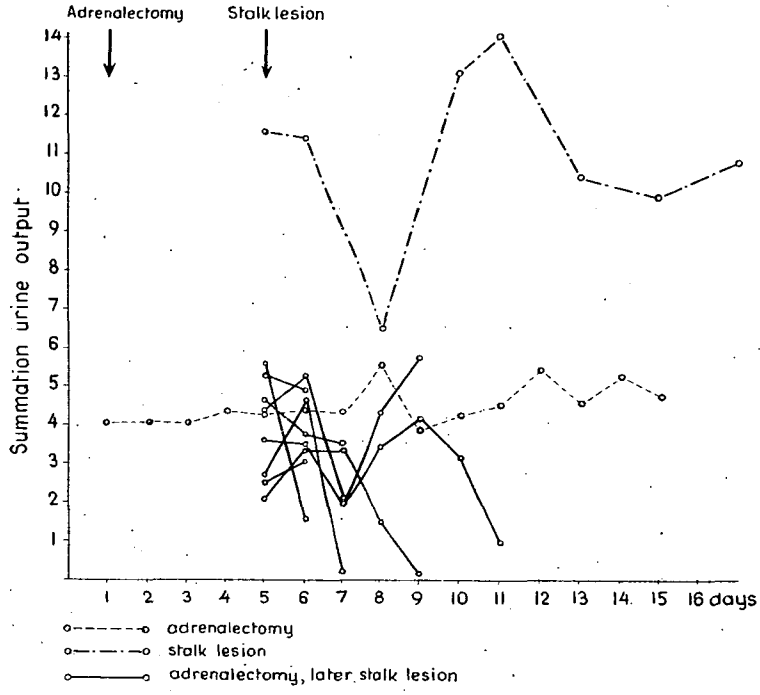


Fig. 40. Summation urine output of rats with adrenalectomy and stalk lesion after administration of physiological NaCl

Considering the above facts is seemed advisable to examine the water metabolism of rats submitted to adrenalectomy and subsequently to stalk lesions after administration of physiological saline. The results are presented on Fig 40. The Fig. shows that the adrenalectomized rats (10 rats) excreted less urine, whereas in the rats with stalk lesions (10 rats) the polyuric reaction was very pronounced. The rats submitted to adrenalectomy and subsequently to stalk lesions only remained alive for a few days after the second operation, thus an individual label had to be applied. In spite of this it could be unequivocally established that polyuria did not develop in any of these animals.

Our investigations indicated that in adrenalectomized rats after oral water loads water retention occurred, however, this was not immediately the case, but only a few days after the surgery. This establishment was in harmony with the observation that the rats bearing stalk lesions also excreted more urine for a few days after the adrenals had been removed, however, this polyuria soon showed a decreasing tendency. If the adrenalectomy was performed earlier, following stalk lesion the amount of

the urine did not increase, whereas by administration of cortisone extensive polyuria could be induced.

Many clinical and experimental observations (GAUNT et al. 1949; GAUNT, 1951; SKILLERN et al. 1956; CHESTER JONES, 1957; PENTZ, 1957; PENTZ et al. 1957; SMITH, 1957; RIBANDO, 1958; REES and ZILVA, 1959; KENNEDY and CRAWFORD, 1961) suggest that following destruction of the stalk, neurohypophysectomy and total body X-ray irradiation, polyuria which would have otherwise developed, failed to occur if the ACTH-adrenocortical system was damaged. However, only a few data could be found indicating after how many days water retention followed the adrenalectomy. The results reported are also contradictory. Most authors (CHESTER JONES, 1957 KENNEDY and CRAWFORD, 1961) found that the enhanced water excretion ceased immediately after removal of the adrenals, others, on the other hand (KAY and ENTENMAN, 1957), observed that the inhibition of the diuresis only ensued after a few days. Our experiments showed unequivocally that for the development of persistent diabetes insipidus in rats with stalk lesions the presence of the adrenals was necessary; however, still 4—5 days after adrenalectomy an enhenced diuretic reaction might occur. In our opinion the results obtained could be very much influenced by the circumstance that most of the investigators measured the water consumption or the daily spontaneous urinary output of the animals, whereas we studied the diuretic reaction following oral water loads. It is obvious, that the results obtained by means of the fairly deviating methods were affected by different factors.

Some authors (CAVALLERO et al. 1954; EDMONDS, 1960) found that by administration of physiological saline the urinary output and daily spontaneous water intake of adrenalectomized rats could be rendered normal. On the ground of this assumption it seemed desirable to repeat the experiment and to give 0,9 or 2 per cent NaCl solutions. But in rats submitted to stalk lesion and adrenalectomy polyuria did not either occur after application of physiological saline. It should also be remarked that in such circumstances the urinary excretion of adrenalectomized rats loaded with 2 per cent NaCl was the same as that of the group loaded with tap water. On the other hand, in the animals to which 0,9 per cent NaCl solution was administered, the only difference observed was that in the second week of the experiment the gradually developing extensive oliguria did not occur, however, the quantity of the excreted urine did not reach the average values found in the intact group.

The data of the literature explain the water retention following adrenalectomy in different ways. Numerous investigators (MARTIN et al. 1939; BIRNIE et al. 1949; KOVÁCS and BACHRACH, 1951; LEAF and MAMBY, 1952; LLOYD, 1952; CAVALLERO et al. 1954; GINSBURG, 1964; MIRSKY et al. 1954 a, b) found that after removal of the adrenals the ADH content increased in the blood and decreased in the hypothalamus and posterior pituitary. Therefore some of them expressed the view that the ADH mechanism played a role in causing the water retention. Whilst others (BURSTON and GARROD, 1952; GARROD et al. 1955; LITTLE et al. 1956; SKILLERN, 1956; CHESTER JONES, 1957) emphasized the importance of factors independent of the antidiuretic hormone (the renal haemodynamical changes due to adrenal hormone deficiency, the decrease of the glomerular

filtration rate, etc.). Our results confirm that ADH cannot play a significant role in the induction of the water retention ensuing after adrenalectomy, as the inhibition of the diuresis also takes place in adrenalectomized rats with stalk lesions and not having any ADH reserve.

2. Function of the adrenal cortex following pituitary stalk lesion

From the aforementioned it is evident that the adrenal cortex influences the water metabolism of the organism to a great extent. That is why on studying the hypothalamo-hypophysial relations of the water metabolism of rats bearing stalk lesions it seemed necessary to extend our investigations to the function of the adrenal cortex. This subchapter deals with the results of these experiments.

Several authors (UOTILA, 1939; CHENG et al. 1949; BARRNETT and GREEP, 1951; MCCANN, 1953, 1957; MCCANN and BROBECK, 1954; FULFORD and MCCANN, 1955; HALÁSZ, 1955, HARRIS, 1955; MOLL and VOGEL, 1959; SMELIK et al. 1959; MCCANN and HABERLAND, 1960; MOLL, 1960) have already studied the functional capacity of the pituitary-adrenocortical axis following pituitary stalk lesion. The results are, however, — obviously owing to the difference of the surgical methods and procedures used to estimate the functional state — contradictory. Some investigators reported that the function of the pituitary-adrenocortical axis had completely caesed, whereas others did not observe an appreciable failure of the function using various methods of investigation. We attempted to examine this question by applying simultaneously many procedures.

The experiments were performed on male and female albino rats of the same strain weighing 150—220 g kept on a standard diet. The stalk was studied some days, and a few cases some weeks after the opeley—Clarke apparatus. After the operation the animals were given tap water ad libitum.

To control the efficiency of the stalk lesion two methods were aplied: on the one hand the water metabolism of the animals was recorded daily and on the other, detailed histological studies of the hypothalamo-hypophysial system were made. For the examination of the function of the adrenal cortex only rats were used in which the pituitary stalk lesion was complete. After both oral tap water, or physiological saline loads all these animals showed characteristic symptoms of diabetes insipidus. The histological methods employed to analyse the water metabolism and to localise exactly the site of the stalk lesion have been described in detail in the previous chapters.

The adrenocortical function of animals bearing pituitary stalk lesions lesions was studied some days, and a few cases some weeks after the operation. Of course, the experiments were also carried out in non-operated control rats.

To determine the functional state of the pituitary-adrenocortical axis the following methods were used:

a. Weighing the adrenals.

The adrenals of the sacrificed animals were removed, carefully

cleaned without being fixed and measured on torsion scales with an accuracy of 0,5 mg. The weight of the two adrenals was computed for 100 g body weight.

b. Histology of the adrenals.

The adrenals fixed in 4 per cent formalin were embedded in paraffin. The sections 4—6 micron thick were stained with haematoxylin-eosin. In some cases frozen sections stained with Sudan III were also made.

c. Determination of the ascorbic acid content of the adrenals.

The left adrenals of the animals were removed under ether anaesthesia, and one hour later the right adrenals were also excised. The estimation of the ascorbic acid content of the adrenals was performed by Roe-Kuether's (1943) 2—4-dinitrophenyl hydrazin method. The ascorbic acid content of the adrenals was computed for 100 g adrenal weight and evaluated.

d. Determination of the corticosterone content of the effluent venous blood of the adrenals.

The cannula was inserted into the vein of the precedingly isolated left kidney of rats anaesthetised with nembutal to which heparin had been administered, and the effluent blood was collected during half an hour. In this period 1 ml Ringer solution was administered to all the rats intravenously to replace the lost blood. The corticosterone was estimated by the method of Weiss and Gláz (1960). The collected blood was centrifuged, extracted with ethyl acetate and evaporated in vacuo at 45° C. The dy substance was dissolved in a little chloroform, dropped on filter paper Whatman No. 1. and run for 5—12 hours in a ligroin:toluol:methanol:water (8,5:1,5:8,0:20,0) system at room temperature. The purpose of this procedure was to purify the substance as the contaminating substances migrate with the moving phase, whereas the corticoids remain on the start line. Subsequently the paper was dried and rechromatographed in a benzol:methanol:water (2:1:1) (Bush B5) system et 45° C. The chromatograms were developed with tetrazolium blue and the yielded formazane was determined quantitatively by means of a Havemann photometer after having been eluated in a mixture of ethylacetate-methanol (7:3). The corticosterone values were expressed as microgram/h/kg body weight.

e. Steroid production of surviving adrenal slices.

The rats were decapitated, the adrenals were immediately removed, weighed on torsion scales and cut into small pieces. The adrenal slices were incubated for 2 hours in an O_2 atmosphere containing 5 per cent CO_2 at 37° C in a Krebs—Ringer bicarbonate solution containing 200 mg per cent glucose, subsequently the steroid content of the media was determined by paper chromatography with the method of Weisz and Gláz (1960) after extraction with aethyl-acetate.. The development was performed with tetrazolium blue. The amount of the formazane formed was measured by means of a Havemann photometer. It should be noted that one estimation was carried out from the adrenals with a total weight of 90—100 mg of 4—5 rats. The steroids were expressed in microgram/h/100 mg adrenal weight.

f. Examination of the changes in the adrenals due to cortisone.

10 mg. cortisone (Adreson, Organon, Oss) was administered daily for 8 days subcutaneously to the rats and the animals were decapitated on the 8th day. The adrenals were immediately removed, fixed in 4 per cent formalin and after having been carefully cleaned weighed on analytical scales with an accuracy of 0,1 mg. The weight of the adrenals was computed for 100 g body weight. The weighed adrenals were embedded in paraffin and 4—6 micron thick slices were made for histological studies. The sections were stained with haematoxilin-eosin.

The biometrical analysis was carried out in all experiments by Student's „t" test.

The results are presented on some Tables.

Table 22. — on which the weights of the adrenals are presented — shows that the weight of the adrenals of female or male rats submitted to stalk lesions 8 days earlier computed for 100 g body weight do not differ substantially from the mean values of the controls. On the other hand, in the female animals 4—5 weeks after the destruction of the pituitary stalk the size of the adrenals diminished significantly; in the male rats with stalk lesions placed at the same time the weight of the adrenals also decreased, however, the difference was not significant as compared to the intact animals.

Histological studies confirmed the results obtained by measuring the weight, i. e. a moderate narrowing of the inner layers of the cortex — the zona fasciculata and reticularis — could be established in rats with atrophied adrenals.

Table 23. indicates the change in the ascorbic acid content of the adrenals following operative stress and unilateral adrenalectomy in the different groups. The results show that the removal of the left adrenals caused a considerable ascorbic acid diminution in the right adrenals of intact rats. The values related to the ascorbic acid content of the left adrenals were on the average about 30—40 per cent lower. The ascorbic acid content of the right adrenals did not decrease appreciably in any of the groups of rats with stalk lesions. In some cases after the left adrenals had been removed ACTH was immediately administered (1,0 mg/rat iv — Cortrophine, Organon, Oss) to rats bearing stalk lesions. After an hour a considerable ascorbic acid depletion ensued in the right adrenals. This observation suggested that in the failure of the adrenal response due to unilateral adrenalectomy and operative stress the inhibition of the ACTH secretion played the primary role and not the decreased reactivity of the adrenals.

Subsequently the corticosterone content of the effluent venous blood of the adrenals was determined. The results may be seen on *Table 24.* It could be established that the adrenals of rats bearing stalk lesions secreted during one hour less corticosterone than those of the intact animals. The decrease of the excreted corticosterone occurred in rats with 8-days-old and in those with 4—5-weeks-old stalk lesions as well, and there was no considerable difference between the two operated groups.

Table 22.

Weight of adrenals calculated for 100 g body weight of intact rats and of rats with pituitary stalk lesion

Group		Time elapsed after operation	Sex	No. of animals	Body weight g	Weight of adrenals mg/100 g body weight	Probability	
I.	Intact	—	♀	16	147,2 ±4,6*	24,4±0,8	I/III.	$p>0,05$
II.	Intact	—	♂	17	187,6 ±6,0	16,0±0,8		
III.	Stalk lesion	8 days	♀	8	187,5 ±3,8	23,9±1,6	I/V.	$p<0,001$
IV.	Stalk lesion	8 days	♂	26	188,5 ±2,0	16,0±0,5	II/IV.	$p>0,05$
V.	Stalk lesion	more than 1 month	♀	11	199,5 ±6,8	18,5±1,4		
VI.	Stalk lesion	more than 1 month	♂	9	182,8 ±7,6	14,3±0,4	II/VI.	$p>0,05$

* Standard error

Table 23.

Effect of operative stress and unilateral adrenalectomy on the adrenal ascorbic acid content of intact rats and of rats with stalk lesion

Group	Time elapsed after operation	No. of animals	Body weight g	Ascorbic acid content (mg/100 g adrenal weight)		Average of differences	Average change in per cent
				Left adrenal	Right adrenal		
Intact	—	12	189,2 ±14,1*	431,0 ±25,0	272,8 ±18,5	—158,3 ± 13,4	63,2
Stalk lesion	2—3 days	7	186,4 ±4,7	426,1 ±35,9	413,6 ±24,3	—12,6 ±15,3	98,3
Stalk lesion	8 days	7	187,1 ±2,6	391,7 ±28,3	424,9 ±25,5	+33,1 ±5,8	109,1
Stalk lesion	more than a month	8	178,8 ±8,5	324,5 ±47,7	295,5 ±36,9	—29,0 ±12,0	93,7

*Standard error

Table 24.

Corticosterone content of the efluent venous blood of the adrenals in intact rats and in rats with pituitary stalk lesion

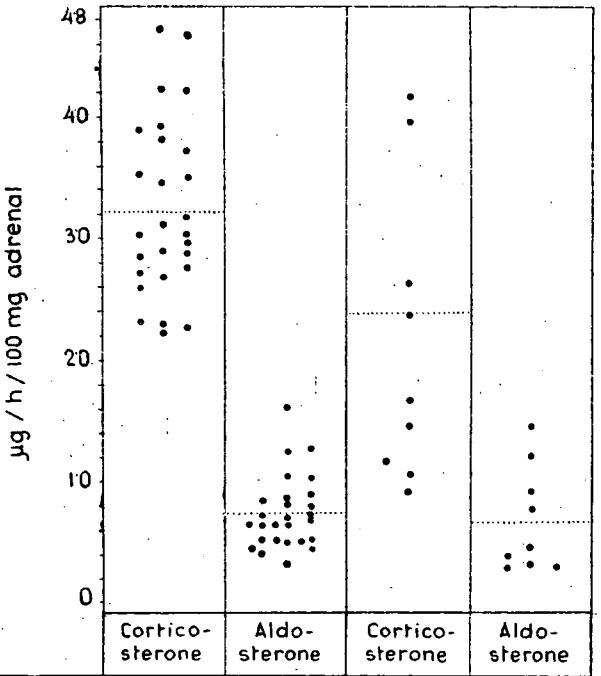
Group	Time elapsed after operation	No. of animals	Body weight g	Corticosterone µg/1 h/kg body weight	Probability	
Intact	—	12	168,8 ±6,0*	105,1 ±3,1		—
Stalk lesion	8 days	10	181,5 ±5,6	50,1 ±6,2	I/II.	$p<0,001$
Stalk lesion	More than a month	16	200,6 ±4,9	41,6 ±5,1	I/III.	$p<0,001$

* Standard error

After the in vivo experiments, the steroid production of the adrenal slices was also studied in vitro. These investigations seemed important because in this way it was also possible to estimate the extent of the aldosterone production. Our method used for the determination of the corticosteroids was not sufficiently sensitive to apply it for the demonstration of the aldosterone contained in the effluent venous blood of the adrenals of an animal. On the other hand, the amount of aldosterone production in the medium of the incubated adrenal slices with a total weight of 80—100 mg obtained from 4, 5 or 6 animals could be well measured by the method applied.

Table 25.

Steroid production of the adrenal slices (in vitro) of control rats and of rats with pituitary stalk lesion



Group	Control		Stalk lesion	
Time elapsed after operation	—		8 days	
Number of experiments	27		9	
Corticosterone	3·23 ± 0·17 *		2·37 ± 0·44	
	0·02 > p > 0·01			
Aldosterone	0·74 ± 0·06		0·66 ± 0·01	
	p > 0·05			

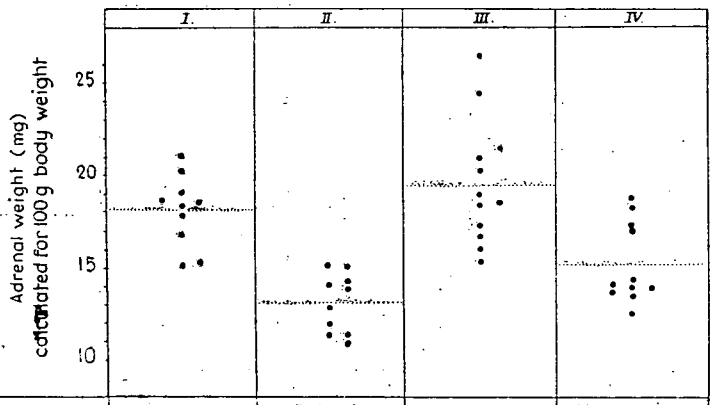
* Standard error

The results of this experiment are illustrated on Table 25. The Table indicates that the adrenals of rats bearing stalk lesions secreted less corticosterone 8 days after the operation. Although the scattering between the different values was fairly pronounced the deviation between the controls and the rats with stalk lesions — in agreement with the data of VAN DER VIES (1960) — was after all statistically significant. There was no considerable difference in the aldosterone production of the two groups.

Finally, the question was investigated whether cortisone administration influenced the weight and tissue structure of the adrenals of rats with 8-old-days stalk lesions. It is known (INGLE and KENDALL, 1937; FARRELL and LAQUEUR, 1955) that this hormone causes a well reproducible atrophy in the adrenal cortex by the inhibition of the ACTH secretion in intact animals. The data of these experiments are presented on Table 26. Our experiments showed that following cortisone treatment the weight of the adrenals computed for 100 g body weight decreased significantly in both intact rats and those having stalk lesions. The results were also confirmed by the histological studies: in rats with stalk lesions treated with cortisone the same extent of atrophy could be observed as in the intact animals — the zona fasciculata and zona reticularis were considerably narrower.

Table 26.

Weight of adrenals after pituitary stalk lesion and cortisone treatment



Group	Intact	Intact, cortisone	Stalk lesion	Stalk lesion, cortisone
Time elapsed after operation	—	—	8 days	8 days
Number of animals	10	10	12	11
Body weight g	194·0 ± 2·9 *	153·5 ± 4·0	146·2 ± 6·5	150·5 ± 9·2
Adrenal weight (mg) calculated for 100 g body weight	18·1 ± 0·6	13·1 ± 0·5	19·5 ± 1·0	15·2 ± 0·7
Probability	—	$^{I}/_{II}$ $p < 0{\cdot}001$	$^{I}/_{III}$ $p > 0{\cdot}05$	$^{III}/_{IV}$ $p < 0{\cdot}01$

* Standard error

One of the most important conclusions which could be drawn from the investigations was that in rats with stalk lesions the application of various methods used for the estimation of the functional state of the hypophysial-andrenocortical axis did not furnish uniform results. Whilst in the adrenals of rats submitted to stalk lesions unilateral andrenalectomy and associated operative stress did not induce ascorbic acid depletion and the corticosterone content of the effluent venous blood of the adrenals, as well as the in vitro corticosterone production decreased significantly as compared to the data of the control group, conversely the weight and the histological structure of the adrenals and the amount of the lipoid demonstrable histologically did not change appreciably, what more, following cortisone administration a pronounced adrenal atrophy developed in rats bearing stalk lesions. After a comparison of the weight and histological picture of the adrenals and the corticosterone production and secretion under other experimental conditions WEISZ et al. (1960) did not observe a parallel change of the parameters examined. *Slusher* (1958) and FISHER and DE SALVA (1959) reached similar conclusions after studying ascorbic acid depletion and corticosterone secretion.

According to our experiments it is difficult to form a definite opinion what factors could explain the difference in the results. It is possible that several kinds of ACTH regulate the adrenal cortex and some of them are further mobilized following the destruction of the stalk. It would be also conceivable that the extension and localization of the pituitary stalk lesion varied in the different animals. The latter assumption may, however, be discarded considering that the region of the hypothalamus and pituitary in all operated rats was studied by histological means, too, and the rats in which the stalk lesion was not complete, were excluded from the evaluation. It seemed the most probable that the reactivity of the adrenal cortex to different stimuli was not identical and that the sensitivity of the various methods differed. The extent of the divergence from the normal values depends on the period and intensity of the stimulus and the inhibition, thus it is evident that the functional differences exerting a shorter and more moderate effect remain masked when less sensitive methods are applied and show values corresponding to the normal averages. At any rate the fact according to which contradictory results were obtained by the parallel application of methods which separately used were adequate for adrenal investigations suggests that one must be very cautious and far-sighted at the estimation of the actual functional state of the adrenal cortex.

However, as a result of our experiments so much could be established that following destruction of the stalk the function of the adrenals changed significantly. Hypofunction developed, which mainly could be correlated with the reduced secretion of ACTH. This concept is supported on the one hand, by the exminations of MCCANN and SYDNOR (1954) who obtained by the parallel application of methods which separately used of rats with stalk lesions (later we will also describe similar data), and on the other, by our own experiments showing that following ACTH administration a significant ascorbic acid depletion ensued in rats with stalk lesions. It should, however, be emphasized that — at least in the period

of the investigation — the function of the adrenal cortex did not cease completely, as a diminished amount of corticoids could also be detected in the effluent venous blood of the adrenals of rats in which the stalk lesion was complete. Our investigations, however, did not elucidate the question to which mechanism this decrease in the function of the hypophysial-adrenocortical system may be attributed. This problem will still be dealt with later.

It seems worthy to mention our observation that cortisone also induced an atrophy of the adrenals in rats with stalk lesions thus this drug also exerted such an effect when the connection between the pituitary and hypothalamus was interrupted. This observation is in harmony with the investigations of CHENG et al. (1949), FORTIER and SELYE (1949), MCDERMOTT (1950) and FORTIER et al. (1957) who showed that certain stimuli could also evoke ACTH mobilization without the presence of direct hypothalamic connections. Others (GANONG and HUME, 1955; HALÁSZ, 1955; ROBERTS and KELLER, 1955; ROSE and NELSON, 1956; ENDRŐCZI et al. 1961; YATES et al. 1961) on the other hand, furnished data that cortisone is also able to exert a direct effect on the anterior pituitary.

Our experiments indicated that in rats with stalk lesions the corticosterone production of the adrenal slices was slightly reduced, whereas the aldosterone production remained substantially unchanged in spite of the fact that when the pituitary stalk lesion was complete diabetes insipidus associated with a pronounced disturbance in the water and electrolyte metabolism developed. The problem of the regulation of the aldosterone secretion will not be dealt with here — in view of the fact that our data are not sufficient for this — it should only be mentioned that this observation which is in good agreement with that of other authors (SWANN, 1940; DEANE et al. 1948; SINGER and STACK—DUNNE, 1955; KOVÁCS et al. 1956; KOVÁCS and DÁVID, 1959) suggests that some partial functions of the adrenal cortex do not react in the same way to certain stimuli.

Chapter IV. analyses the question why the enhanced diuretic reaction developing after water loads ceases in rats submitted to stalk lesion several weeks earlier. On examining the function of the adrenal cortex in this period the hypofunction of the hypophysial-adrenocortical axis could be established also some weeks after the operation. This functional disturbance might induce the diminution of the diabetes insipidus. On the other hand, the fact that the increased diuresis only ceased after some weeks, whereas the reduction of the functional capacity of the hypophysial-adrenocortical axis already ensued shortly after the destruction of the pituitary stalk, furthermore that in the animals suffering from a temporary diabetes insipidus the functional disturbance of the adrenal cortex was not more severe suggest that other factors play an important role in the regression of diabetes insipidus after several weeks following destruction of the pituitary stalk.

3. Changes in the pituitary and adrenal cortex following oestrogen hormone treatment in rats with hypophysial stalk lesions

It is known that in rats oestrogen hormone administration resulted in the enlargement of the adrenals, a broadening of the cortical layers and decrease in corticosterone secretion. (VOGT, 1955, 1957; HOLZBAUER, 1957; TELEGDY et al. 1962; SABA and HOET, 1963; PINCUS and HIRAI, 1964). According to VOGT (1955) and HOLZBAUER (1957) oestrogen hormone inhibits corticosterone production. This causes owing to the effect of the negative feed back mechanism an enhanced ACTH secretion and as a result of the great amount of ACTH released the adrenals became enlarged. This assumption was supported by the observations showing that the adrenal hypertrophy due to oestrogen administration did not develop if the animals were previously hypophysectomized or submitted to cortisone treatment (SELYE and COLLIP, 1936; KOVÁCS et al. 1958, 1960; DÖRNER and HOHLWEG, 1961). Thus, the changes in the adrenals caused by oestrogen hormone seemed suitable for studying the regulation of the ACTH secretion.

A previous subchapter already dealt with similar questions and it was demonstrated that cortisone also induced atrophy of the adrenal cortex in rats with stalk lesions. Therefore it seemed interesting to examine how the destruction of the pituitary stalk influenced the changes which developed in the pituitary and adrenals following oestrogen treatment. In the present subchapter we report the results of these experiments.

The experiments were performed in female albino rats from the same strain weighing 120—220 g kept on a standard diet. The animals were divided into 4 groups. The first group served as control. These animals were not treated and not operated on. The rats of the second group were treated with oestrogen, but no surgical manipulation was performed. The rats of the third group were submitted to pituitary stalk lesion. These animals were not treated with injections. The fourth group consisted of rats bearing lesions of the stalk. Oestrogen hormone was also administered to them.

The oestrogen hormone treatment lasted 10 days. 1,0 mg oestrone acetate (Hogival, Chinoin) was injected once daily subcutaneously.

The destruction of the stalk was performed under nembutal anaesthesia by means of a Horsley—Clarke apparatus. The manner of the localisation of the lesion was already described in detail previously. The water metabolism of the animals was also controlled. For the experiment only rats in which the diabetes insipidus was pronounced and the stalk lesion was complete, were used. Some of the rats bearing stalk lesions (group 4.) also received oestrogen hormone: The treatment was initiated 4 weeks after the operation and lasted 10 days. Then the animals were killed. The rats with lesions of the stalk, which were not treated with injections, were sacrificed simultaneously.

The animals were decapitated. Subseuqently they were immediately autopsied and the organs fixed in 4 per cent formalin, and the weight of the cleaned organs was measured on analytical scales with an accuracy

of 0,1 mg. The values were computed for 100 g body weight. The pituitaries of the rats were fixed in Susa solution and embedded in paraffin. The 4—6 micron thick serial sections were stained by Goldberg—Chaikoff's trichrom staining. Gomori's chromalum-haematoxylin, Gomori's aldehyde-fuchsin, Paget—Eccleston's aldehyde-thionin and also by PAS reaction. The organs were embedded in paraffin and 4—6 micron thick sections were prepared and stained with haematoxylin-eosin. Serial sections were made of the pituitaries and adrenals.

The nuclei of the chromophobe cells contained in the anterior pituitary and the nuclei of the cells of the three layers of the adrenal cortex were also studied by means of the karyometric method in sections stained with haematoxylin-eosin after having been fixed in formalin. The two diameters of the nuclei of 100 chromophobe cells of the anterior pituitary and those of 100 cells contained in the zona glomerulosa, fasciculata and reticularis of the adrenals were determined in each animal by the „two axis method", and their cubic volume was calculated by the rotation ellipsoid formula $\frac{4ab^2\pi}{3}$. Concerning the details of the karyometric analysis we refer to a previous paper (DÁVID et al. 1957). The results were evaluated biometrically by Student's „t" test.

The results are presented on Tables. On Table 27. the average weights of the different organs are listed computed for 100 g body weight. The Table shows that the pituitary weight of intact animals treated with oestrogen hormone increased. The adrenals also became moderately enlarged, whereas corresponding to our previous investigations (KOVÁCS et al. 1958; DÁVID and KOVÁCS, 1962) and to the data of the literature (HERTING and SATHE—EICHLER, 1955; SELYE et al. 1955; SELYE and MARION, 1955; WEAVER, 1955; DÖRNER and HOHLWEG, 1961) the weight of the thymus decreased. The weight of the ovaries increased. The weight of the uterus rose also considerably; this change indicated that an effective oestrogen preparation was used. In rats with one month-old lesions of the pituitary stalk the loss of weight of the pituitary which could also be proved biometrically, was striking. In this group the size of the adrenals and ovaries diminished, whereas the weight of the thymus did not change appreciably. The uteri became smaller, too, however, this alteration was statistically — owing to the considerable scattering — not significant as compared to the control values. In rats with stalk lesions subsequently treated with oestrogen hormone the weight of the pituitaries did not reach that found in the intact rats after oestrogen hormone administration, but was significantly greater than that of the untreated animals bearing pituitary stalk lesions. In the operated animals following oestrogen hormone treatment the weight of the adrenals, ovaries and uteri rose, whereas that of the thymus diminished. It should be mentioned that the enlargement of the adrenals and ovaries was significant related to the rats in which only the pituitary stalk had been destroyed without administration of oestrogen; however, the increase in weight did not reach the level observed in intact rats after oestrogen treatment.

Histological studies of the organs confirmed the results of the measurements. Only the more important changes will be mentioned. In the pi-

Table 27.

Effect of oestrone administration on the weight of the organs calculated for 100 g body weight of intact rats and of rats with stalk lesion

Group		No. of animals	Body weight g	Pituitary mg	Adrenal mg	Thymus mg	Ovary mg	Uterus mg
I.	Control	11	185,8±3,5*	7,1±0,2	33,2±1,1	104,2±9,9	49,8±2,3	242,0±14,0
II.	Oestrone	11	179,1±6,9	10,0±0,5	37,0±1,3	72,6±9,4	67,6±3,9	405,6±119,2
III.	Stalk lesion	10	178,5±3,1	4,4±0,2	27,0±1,9	116,2±14,0	32,4±2,4	199,5±21,3
IV.	Stalk lesion+ oestrone	10	172,6±5,9	6,7±0,5	31,0±1,3	66,3±6,6	53,6±3,0	367,6±39,5
Probability:	I/II.			$p<0,001$	$0,05>p>0,02$	$0,05>p>0,02$	$p<0,001$	$p<0,05$
	I/III.			$p<0,001$	$p<0,01$	$p<0,05$	$p<0,001$	$p<0,05$
	II/IV.			$p<0,001$	$p<0,01$	$p<0,05$	$p<0,01$	$p<0,05$
	III/IV.			$p<0,001$	$p<0,05$	$p<0,01$	$p<0,001$	$p<0,01$

*Standard error

tuitaries of the rats treated with oestrone a hyperplasia of the chromophobe cells could be detected and the degranulation of the basophilic cells could be established. The histological picture was in good agreement with that described in previous papers (Kovács et al. 1958a; DÁVID and Kovács, 1962a). The adrenal cortex seemed broader. The cells of the zona fasciculata were moderately enlarged. In the thymus a reduction of the lymphoid elements could be seen. Very striking alterations could be observed in the pituitaries having diminished in size of the rats with lesions of the stalk. The posterior pituitary had atrophied to a great extent, its substance seemed rich in cells. Neurosecretory granules could not be demonstrated by staining with Gomori's chromalum-haematoxylin, Gomori's aldehyde-fuchsin and Paget—Eccleston's aldehyde-thionin. Similar observations were reported as a result of previous examinations (Kovács et al. 1963), too. The intermediate lobe seemed broader in some of the animals. This difference was, however, not pronounced and did not prove to be significant according to earlier volumetric examinations (LÁSZLÓ et al. 1962d). The anterior pituitary of rats operated one month before was also considerably smaller. The most striking change in the anterior lobe was that in the middle of the gland a connective tissue scar rich in fibres and poor in cells was present. In this area the glandular parenchyma was completely destroyed. The central scar was, as pointed out in previous papers (LÁSZLÓ et al. 1962d; DÁVID et al. 1965), due to the organisation and atrophy of a previously far more extensive ischaemic necrosis. It is known that following destruction of the pituitary stalk the portal vessels get damaged and a serious disturbance of the blood supply of the anterior lobe ensued. The central part of the anterior lobe became necrotic owing to hypoxia; a typical ischaemic infarction developed there. In rats submitted to destruction of the stalk in the preceding weeks only a central scar could be seen, necrosis was no more present. As under normal conditions the tissue of the anterior pituitary is not able to regenerate appreciably (KOVÁCS, 1961) the volume of the anterior pituitary becomes considerably smaller. Around the central scar the surviving glandular substance could be recognised. Here the cells seemed intact. In this experimental series quantitative cytological examinations were not performed, thus the ratio of the different kinds of cells could not be expressed in numbers. However, it could be established that in addition to the chromophobe cells basophilic and eosiniphilic ones were also present. This observation seemed to contradict the recent data of DANIEL et al. (1964) who observed in rats with stalk lesions the gradual decrease of the number of the basophilic cells in the surviving parenchyma. It should, however, be emphasized that our data could hardly be compared with the results of ANIEL et al. 1964) as in our case the pituitary stalk was destroyed stereotaxically and the regeneration of the portal veins of the stalk was not prevented, they, on the other hand, destroyed the stalk by surgery and prevented the regeneration of the portal vessels of the pituitary stalk by inserting a foreign substance between the stumps of the stalk, i. e. inhibited the re-establishment of the direct vascular connections between the hypothalamus and anterior pituitary. The study of other organs of rats bearing stalk lesions showed that in the endocrine organs atrophy, varying

in extent, developed. In the adrenals the cortical substance became narrower. The cortical cells seemed to be smaller. This establishment concerned the cells of the zona fasciculata and reticularis. The zona glomerulosa was rather broader than normally. In the single animals the atrophy varied within fairly wide limits. In some of the rats the atrophy was very pronounced. These adrenals were similar to those of the hypophysectomized rats. In other animals only a slight atrophy could be found. In the ovaries of most of the animals a moderate hypofunction could be noted. Apparently there was also atrophy in the uterus. The thymus did not show an appreciable change.

In the organs of rats with lesions of the stalk significant alterations appeared after oestrogen hormone treatment. In the anterior pituitary of the rats belonging to this group a tiny central scar was found, but the surviving parenchyma seemed broader. Quantitative cell counts were not performed, yet in our opinion the basophilic cells were less in number and their cytoplasm was markedly degranulated. Big chromophobe cells dominated the picture. The intermediate lobe and the posterior lobe were similar to those found in rats to whom oestrogen hormone had not been administered and whose pituitary stalks had been destroyed in the preceding weeks. The pars intermedia seemed slightly broader, whereas the posterior pituitary was considerably atrophied. The posterior lobe did not contain neurosecretory substance. In these rats the atrophy of the adrenal cortex was not pronounced, what more, the cortical layers seemed broader as compared to those of the untreated rats with pituitary stalk lesions. The thymus and gonads showed alterations like those seen in the intact rats treated with oestrogen hormone.

The qualitative histological changes found in the pituitaries and adrenals of the oestrogen hormone treated rats seemed to merit karyiometric analyses of the chromophobe cells of the pituitary and the cells contained in all three layers of the adrenal cortex in all experimental groups. The results are presented in Table 28. and 29.

Table 28. illustrates the average cubic volume of the nuclei of the chromophobe cells in the anterior pituitary in the different experimental groups. It can be seen in the Table that the average cubic volume of the nuclei of the chromophobe cells in oestrogen hormone treated intact rats was significantly higher than that of the untreated controls. The deviation between the two groups is also biometrically significant. In rats submitted to destruction of the stalk an appreciable alteration did not occur as compared to the average values of the controls. In rats in which the lesion of the stalk had been inflicted some weeks earlier the cubic volume of the nuclei of the chromophobe cells increased significantly following oestrogen hormone treatment. The increase of the volume did not reach that of the oestrogen treated intact rats, but it could be recorded by a comparison of the values found in oestrogen treated rats with stalk lesions with those of rats bearing stalk lesions, but not having received oestrogen treatment.

Table 29. indicates the changes in the cubic volume of the nuclei measured in the different layers of the adrenal cortex in the various experimental groups. It may be established from the Table that between the single experimental groups considerable shifts ensued in the average

Table 28.

Effect of oestrone administration on the nuclear volume ot chromophobe cells of the adenohypophysis of intact rats and of rats with stalk lesion

Group		No. of animals	No. of cells	Nuclear volume of choromophobe cell 7^3	Probability	
I.	Control	8	800	77,6±1,4*	I/II.	$p<0{,}001$
II.	Oestrone	9	900	134,7±5,3	I/III.	$p>0{,}05$
III.	Stalk lesion	8	800	81,3±4,0	II/IV.	$p<0{,}01$
IV.	Stalk lesion + oestrone	9	900	112,6±2,7	III/IV.	$p<0{,}001$

* Standard error

Table 29.

Effect of oestrone administration on the volume of the cell nuclei of the adrenal cortex of intact rats and of rats with pituitary stalk lesion

Group		No of cells in the different layers	Volume of cell nuclei in the different layers of the adrenal (μ^3)		
			Glomerulosa	Fasciculata	Reticularis
I.	Control	800	60,7±2,8*	121,9±2,2	86,1±2,7
II.	Oestrone	800	55,9±2,8	146,2±4,2	95,9±4,8
III.	Stalk lesion	800	73,3±5,1	109,9±3,3	82,3±1,4
IV.	Stalk lesion + oestrone	800	70,2±3,4	131,0±3,5	91,3±2,8
* Standard error					
Probability:	I/II.		$p>0{,}05$	$p<0{,}001$	$p>0{,}05$
	I/III.		$p>0{,}05$	$p>0{,}01$	$p>0{,}05$
	II/IV.		$p<0{,}01$	$0{,}02>p>0{,}01$	$p>0{,}05$
	III/IV.		$p>0{,}05$	$p<0{,}001$	$p<0{,}01$

cell cubic volume of the nuclei expressed in cubic microns. In the zona glomerulosa oestrogen treatment did not induce an appreciable alteration; the average values of the nuclear cubic volumes of the cells did not show any remarkable deviation from those of the controls. In rats submitted to destruction of the stalk, on the other hand, a moderate enlargement of the nuclear cubic volume of the cells occurred. This change was independent of the fact whether or not oestrogen treatment was applied; it could be observed in both the treated and untreated rats with stalk lesions. It should be mentioned that our data are in harmony with the reports of ENDES et al. (1959) who observed the broadening of the zona glomerulosa in rats bearing stalk lesions. Following oestrogen treatment the nuclear cubic volume of the cells in the zona fasciculata increased in intact animals. In the untreated rats with pituitary stalk lesion an the other hand, the nuclear cubic volume of the cells decreased significantly. This deviation is in good agreement with the results of the weight measurements and qualitative histological studies of the adrenals. The findings obtained in oestrogen treated rats bearing lesions of the stalk seemed interesting. In this group a significant enlargement of the nuclear cubic volume of the cell could be detected after a comparison of the data with those of the untreated rats with pituitary stalk lesions. This experimental series suggested that the oestrogen hormone was able to affect the adrenals of rats having stalk lesions, too. In rats bearing stalk lesions the average nuclear cubic volume of the cells of the zona reticularis did not deviate substantially from the values of the untreated intact rats. Apparently, administration of oestrogen hormone also caused an increase in the nuclear cubic volume of the cells in the zona reticularis. In intact rats this alteration was owing to the greater scattering between the two groups not significant. A considerable difference in the values of the average nuclear cubic volume of both treated and untreated rats with stalk lesions could be established; the nuclear cubic volume of operated rats increased following oestrogen hormone treatment.

Our examinations showed that administration of oestrogen hormone influenced significantly the structure of the pituitary and adrenals. The weight of the pituitary of intact animals rose, the basophilic cells in the anterior lobe degranulated and the chromophobe cells dominated. The nuclear cubic volume of the chromophobe cells increased significantly. The adrenals became visibly hyperthrophic, the cortical substance broadened. The nuclear cubic volume of the cells in the zona fasciculata became enlarged. The alterations in the pituitary and adrenals due to oestrogen hormone also did not only develop in the intact animals, but in rats with one month-old stalk lesions, too. Although the parameters examined in the operated animals did not reach those found in oestrogen hormone treated intact rats, yet they exceeded considerably the average values observed in untreated rats bearing pituitary stalk lesions. Thus, it is beyond doubt that oestrogen hormone could exert its influence on the pituitaries and adrenals of rats submitted to stalk lesions a month previously.

After obtaining these results, of course, the question arose how oestrogen hormone induced the changes observed in the pituitary and adrenals. Before drawing any conclusions concerning the mode of action

of the oestrogen hormone some properties of the endocrine system of the rats with pituitary stalk lesions used for the experiments ought to be mentioned which have already been dealt with previously. The destruction of the stalk evoked a damage of the portal vessels, the complete interruption of the neural connections between the hypothalamus and the neural lobe, the ischaemic necrosis of the anterior lobe and an extensive atrophy of the posterior lobe. One month after the operation owing to the regeneration of the portal vessels the direct vascular connection between the hypothalamus and the anterior pituitary was restored. The blood flow of the pituitaries in rats operated in the preceding weeks was also found by means of the very sensitive and reliable Sapirstein's Rb radioactive method to be normal (DÁVID et al. 1965). In spite of the regeneration of the portal vessels, the normal blood flow and the restoration of the direct hypothalamo-hypophysial vascular connections the function of the anterior pituitary was not adequate. This is proved by the fact that the endocrine organs of rats with some-weeks-old lesions were moderately atrophied and thus a close parallelism between the function of the portal vessels and that of the anterior pituitary could not be found.

To return after the above discussion to the question of the mechanism of the changes developing in the pituitary and adrenals following oestrogen hormone administration, in our opinion the changes observed in the pituitary — the enlargement of the pituitary, the degranulation of the basophilic cells and the increase in the nuclear cubic volume of the chromophobe cells — developed as a result of the direct effect exerted by the oestrogen hormones on the parenchyma of the pituitary. Other data also supported the fact that the oestrogen hormone could exert an influence on the parenchyma of the pituitary independently of the hypothalamus. In previous investigations it has been demonstrated (JULESZ et al. 1965) that after the anterior pituitary was transplanted into the anterior chamber of the eye owing to which it was surely devoid of the direct hypothalamo-hypophysial vascular connections it increased in size following oestrogen hormone administration. Tumours also formed in the anterior pituitary grafted into the anterior chamber of the eye and under the kidney capsule if the animals were treated over an adequate period with suitable doses of oestrogen hormone (CLIFTON and FURTH, 1961; JULESZ et al. 1965). In adenohypophysial autografts also containing basophilic cells of cortisone treated rats oestrogen hormone administration induced basophilic degranulation (KOVÁCS et al. 1958, 1960). FLERKÓ and BÁRDOS (1960) reported an enlargement of the pituitary in rats with lesions of the hypothalamus which developed a state of permanent oestrus as a result of an enhanced secretion of oestrogen hormone. BOGDANOVE (1963), ROIE and NELSON (1957) also suggested that the oestrogen hormone exerted owing to its direct hypophysial effect an influence on the structure of the anterior pituitary lobe. In a previous experiment (DÁVID and KOVÁCS, 1962a) it was found that the morphium treatment applied to induce a pharmacological blockade of the hypothalamus could not prevent the increase of the size of the pituitary due to oestrogen hormone, as well as the degranulation of the basophilic cells.

It is very difficult to explain the mechanism of the changes developing in the adrenals after oestrogen hormone treatment. Three possibilities can be suggested. The first is that the oestrogen hormone exerts its direct effect on the adrenals inhibiting their corticoid production. Thus, owing to the diminished corticoid level ACTH hypersecretion ensues resulting in hypertrophy of the adrenals. If this possibility holds good oestrogen hormone plays only an indirect role in bringing about the enlargement of the adrenals and our experiments must offer an explanation how owing to the lack of corticosterone ACTH mobilization develops in animals with lesions of the pituitary stalk. Although, great importance is attributed to the effect of the inhibition of corticoid production which the oestrogen hormone exerts directly on the adrenals, it seemed questionable and even improbable that this mechanism could play a decisive role in the development of the adrenal hyperthrophy caused by oestrogen treatment of rats whose pituitary stalk had been destroyed. In rats operated more than a month previously the activity of the pituitary adrenocortical axis also decreased without any treatment. This is proved by the facts that the weight of the adrenals diminished significantly, a histological picture suggesting hypofunction could be observed and using the method of direct corticosterone determination it could established that the adrenals produced less corticosterone than normally. Thus, the effect of the oestrogen hormone inhibiting corticoid production causing an enhanced ACTH secretion as a result of the elimination of the feed back mechanism could hardly come into play in rats with stalk lesions having already a diminished hypophysio-adrenocortical capacity. It is obvious that if the pituitary of rats with stalk lesions and adrenocortical hypofunction would be able to react suitably to the corticoid lack, following destruction of the stalk, instead of atrophy of the adrenals, hypertrophy ought to be found, without application of oestrogen treatment, too.

The second possibility with which the adrenal hypertrophy inducing effect of oestrogen could be explained is that oestrogen hormone administration renders the adrenals sensitive to ACTH. This assumption seems improbable because in rats with pituitary stalk lesions ACTH hyposecretion is present, thus the enlargement of the adrenals can hardly be due to the peripheral synergism prevailing presumably between the oestrogen hormone and the ACTH on the adrenal level. The results of previous investigations (DÁVID et al. 1957) showing that in oestrogen treated rats ACTH administration only increases corticosterone production to a slight extent as compared to that of the controls, contradict this hypothesis.

The third possibility would be that the direct effect exerted by the oestrogen hormone on the hypothalamo-adenohypophysial system enhances ACTH secretion. This hypothesis is also supported by some data of the literature. It is known that following oestrogen treatment the ACTH content of the pituitary decreases, whereas that of the blood increases (HALMI and BOGDANOVE, 1951; GEMZELL, 1952). In certain circumstances the intrahypothalamic implantation of the oestrogen hormone induces hypertrophy of the adrenals (KANEMATSU and SAWYER, 1963). Oestrogen hormone administration causes a significant change in O_2 consumption of the anterior pituitary (GAULL and VILLEE, 1959). These

observations suggest that the enlargement of the adrenals could be due to the central effect exerted by the oestrogen hormone on the hypothalamo-hypophysial system. Our examinations have, however, not elucidated whether the oestrogen hormone exerts its effect inducing ACTH mobilization on the glandular cells of the anterior lobe, or on the hypothalamic centres. Our experiments performed in rats with pituitary stalk lesions do not promote the solution of this problem as owing to the regeneration of the portal vessels the direct hypothalamo-hypophysial vascular connections became restored. It could also be assumed that the oestrogen hormone induces ACTH mobilization and thus a hypertrophy of the adrenals by its direct effect exerted on the anterior pituitary, or by an indirect effect on the hypothalamic centres. If the oestrogen hormone exerts its ACTH hypersecretion inducing effect via the hypothalamic centres, it may be that the oestrogen hormone increases the hypothalamic neurohumor (CRF) production responsible for ACTH mobilization, or abolishes the inhibition of the mobilization caused by the destruction of the pituitary stalk. These questions can only be elucidated by further investigations.

4. Adrenocortical function in the different phases of diabetes insipidus

A study of the correlation between the hypothalamo-hypophysial system and the water homeostasis showed in previous investigations that the destruction of the pituitary stalk induced a characteristic, polyphasic disturbance in the water metabolism. Immediately after the operation polyuria and polydipsia occurred, then the water consumption of the rats decreased, the specific gravity and the electrolyte concentration of the urine increased, and after oral water loads a diuretic reaction did not develop, i. e. water retention could be observed. This antidiuretic phase began on the third or fourth postoperative day and lasted for 1—2 days. Following the antidiuretic phase urinary output and water consumption increased; characteristic diabetes insipidus developed.

On the basis of the results described in Chapter III. it seemed reasonable to draw the conclusion that in rats with destruction of the stalk the oliguric phase was in connection with the antidiuretic hormone which was discharged into the circulation, i. e. the hypersecretion of the antidiuretic hormone released from the hypothalamo-neurohypophysial system damaged as a result of stalk lesion was the cause of the transient water retention.

As the administration of antidiuretic hormone causes under varying experimental conditions a release of ACTH thus playing according to several authors (McCann and Brobeck, 1954; McCann and Sydnor, 1954; Mirsky et al. 1954; McCann, 1957; Martini et al. 1960; Casentini et al. 1961; De Wied, 1961) a significant role in the regulation of ACTH secretion it seemed worth while to examine further whether in rats with lesions of the stalk a certain correlation between the momentary state of the water metabolism and the function of the pituitary adrenocortical axis could be demonstrated. To estimate the function of the adrenal

cortex the determination of the corticosterone content of the plasma and the measurement of the steroid formation of the surviving adrenal slices was used. If the ADH is actually the hypothalamic neurohumoral mediator substance responsible for the ACTH release then during the oliguric phase associated with excessive ADH mobilization hypercorticosteronaemia and correspondingly an enhanced in vitro corticosteroid production ought to be seen in rats bearing lesion of the stalk.

The experiments were performed on female albino rats from the same strain weighing 160—180 g and kept on a standard diet. The destruction of the stalk was carried out under nembutal anaesthesia by means of the Horsley-Clarke stereotaxic apparatus. Following surgery the water metabolism of the animals was studied repeatedly and steroid determinations were only accomplished in rats with typical disturbances of the water metabolism, and in those which according to histological studies performed as described in Chapter II. had total lesions of the stalk. The animals not corresponding to the above criteria were excluded from the evaluation.

The determination of the corticosterone content of the plasma was carried out by the method of VECSEI—WEISZ and KEMÉNY (1963). The animals were exsanguinated by decapitation. The blood was kept in test-tubes containing heparin. The combined blood of two animals was used for one determination. The steroids were extracted from the plasma to which heparin had been added with chloroform. The extracts were chromatographed in a BUSH *B* 5 system (1952) after having been submitted to purifying chromatography according to WEISZ and GLÁZ (1960). Following development with tetrazolium the formazane formed was determined quantitatively by means of an Unicam 500 spectrophotometer at a wave length of 530 millimicrons.

The determination of the steroid formation of the surviving adrenal slices was performed by the method of VAN DER VIES et al. (1960). The rats were sacrificed by decapitation and the adrenals were removed, subsequently the cleaned adrenals were weighed and quartered. The slices were put into flasks containing 2 ml of Krebs—Ringer bicarbonate-glucose (200 mg%) solution and shaken in a Dubnoff metabolic incubator at 37° C in a 95 per cent O_2 atmosphere containing 5 per cent CO_2. Total corticoids in the medium were extracted with methylene chloride and determined by means of a Zeiss spectrophometer at a wave length of 240 millimicron.

The results were evaluated biometrically with Student's „t" test and recorded on tables.

Table 30.

Plasma-corticosterone content of non-operated control rats and rats with pituitary stalk lesion

Group		Time elapsed after operation	No. of cortisocterone determinations	Plasma corticosterone µg per cent**	Probability
I.	Non-operated control	—	10	80,0±11,1*	—
II.	Stalk lesio :	2 days	7	41,2±8,3	I/II. $0,05>p>0,02$
III.	Stalk lesion	3—4 days	9	45,3±6,2	I/III. $0,02>p>0,01$
IV.	Stalk lesion	more than 2 months	10	41,1±7,5	I/IV. $p<0,01$

*Standard error

**The plasma of two rats was used for one determination

Table 30. shows that in polyuric rats submitted to pituitary stalk lesions two days, or more than three weeks previously, the corticosterone content of the plasma indicates a significant decrease related to the values of the non- operated controls. A similar extent of diminution could, however, also be seen in oliguric rats with stalk lesions performed three or four days earlier, although these animals secreted excessive amounts of antidiuretic hormone and retained water.

Investigations of the in vitro steroid production (Table 31) showed that the hypofunction of the adrenal cortex could be demonstrated by means of this parameter, too, in animals bearing destructions of the stalk. This divergence was also considerable in the interphase and only the steroid production of rats with chronic lesions showed a rising tendency, however, this value was also significantly below the values of the controls.

Before and after the oliguric interphase the reduced corticosterone level observed in polyuric rats suffering from diabetes insipidus and the in vitro corticosteroid production were substantially in harmony with the results of previous investigations and with the data of the literature (Barrnett and Greep, 1951; Greep and Barrnett, 1951; Harris, 1955a; Munson and Briggs, 1955; Martini and De Poli, 1956) suggesting that after the interruption of the direct hypothalamo-hypophysial connections a hypofunction of the hypophysial-adrenocortical axis ensued. It is known that in animals with lesions after unilateral adrenalectomy compensatory

Table 31.

Total corticosteroid production (in vitro) of adrenal slices of non-operated control rats and of rats with pituitary stalk lesion

Group		Time elapsed after operation	No. of animals	Total corticosteroid production µg/100mg adrenal/h	Probability	
I.	Non-operated control	—	12	25,5±1,3*		—
II.	Stalk lesion	1 day	8	11,0±0,8	I/II.	$p \ll 0{,}001$
III.	Stalk lesion	3 days	10	9,1±0,9	I/III.	$p \ll 0{,}001$
IV.	Stalk lesion	5 days	8	11,1±1,3	I/IV.	$p \ll 0{,}001$
V.	Stalk lesion	7 days	11	11,7±0,9	I/V.	$p \ll 0{,}001$
VI.	Stalk lesion	14 days	11	12,9±1,0	I/VI.	$p \ll 0{,}001$
VII.	Stalk lesion	more than 1 month	10	14,5±0,9	I/VII.	$p < 0{,}001$

* Standard error

hypertrophy and ascorbic acid depletion did not take place and the corticosterone content of the effluent venous blood of the adrenal decreased, as well. The present investigations represent an advance as far as at the analysis of the functional capacity of the hypophysial-adrenocrtical axis of rats bearing stalk lesions the changes in the water metabolism were taken into consideration, as well.

Our experiments did not only deal with the function of the adrenal cortex in polyuric rats, but also with that of the animals having transitory water retention, i. e. when pathologic hypersecretion of ADH in excessive amounts took place. It seemed remarkable that neither the corticosterone level of the plasma nor the total corticosteroid production of the surviving adrenal slices rose in such animals, what more, in spite of the enhanced endogenous ADH mobilization, values were found suggesting a marked hypofunction of the adrenal cortex as compared to those of the controls.

According to our experiments the evident conclusion could be drawn that in such cases there was no close parallelism between the extent of ADH secretion and the activity of the hypophysial-adrenocortical axis.

Several data indicate the role played by vasopressin in ACTH mobilization: following stress the release of ACTH changed parallel with the adiuretin secretion and the depletion of the neurosecretory material contained in the hypothalamus and neurohypophysis (ROTHBALLER, 1953, 1956; SCHARRER and FRANDSON, 1954); small amounts of ADH enhanced ACTH release (MCDONALD and WEISE, 1956 a, b; SAWYER, 1961; GRINDELAND and ANDERSON, 1963); the effect of vasopressin to increase ACTH mobilisation could be demonstrated in animals submitted to corticoid-, nembutal- or morphium inhibition (MCCANN, 1957), as well as in animals with a damaged median eminence (MCCANN, 1957; DE WIED, 1961 a, b); after ADH treatment a rise of corticoid production was shown in animals with a pituitary graft (CHAUVET and ACHER, 1959; MARTINI et al. 1959). SAFFRAN (1959) reports similar observations on studying the problem in vitro. In view of these data the question arises why unlike exogenus vasopressin the endogenus hormone released in the course of the antidiuretic phase from the hypothalamo-neurohypophysial system is not able to induce ACTH mobilization in rats with pituitary stalk lesion? Our investigations did not provide an answer to this question; however, in our opinion the following possibilities may offer an explanation:

1) the effect of exogenous or endogenous ADH exerts on the pituitary is not identical; 2) owing to the extensive central necrosis developing after stalk lesion the pituitary does not contain sufficient amounts of ACTH; 3) the sensitivity of the anterior pituitary to endogenous ADH is diminished; 4) the endogenous ADH of rats with destroyed stalks does not reach the glandular cells of the anterior pituitary; 5) in the oliguric phase not only ADH is released from the hypothalamus- neurohypophysis of rats bearing stalk lesions but also some substance inhibiting the effect of ADH on the mobilization of ACTH; 6) ADH is not identical with factor causing ACTH release.

Only further examinations can elucidate which of these assumptions is the right one.

5. *Investigations on the functional correlation between the ADH and ACTH systems*

In the experiments described in the previous parts it has been established that in rats with lesions of the stalk the large amount of adiuretin released during the oliguric phase did not influence significantly the function of the ACTH-adrenocortical axis. The exact cause of this phenomenon, could, however, not be explained only a few assumptions were suggested. Further examinations are needed for the resolution of the problem. This subchapter gives an account of the results.

Five hundred sixty-one male albino rats of the same strain weighing 180—280 g kept on a standard diet were used. Pituitary stalk lesions were made while the rats were anaesthetized with ether by means of a modified Krieg stereotaxic apparatus (BOUMAN et al. 1957). The description of the operation and the histological studies are reported in detail in Chapter II. Hypophysectomy was performed via the transauricular route with the technique of KOYAMA (1931) and at the end of the experiment the region of the sella was carefully examined macroscopically.

The functions of the adrenal cortex were studied by determining the in vitro steroid formation of the adrenal slices as described previously (VAN DER VIES et al. 1960).

To provoke ACTH release, rats were exposed to a strange environment by transferring the animals from the ratroom to the operating room. Half an hour later they were anaesthetized with ether and the left adrenal gland was removed. Another half an hour later the rats were decapitated and the right adrenal gland was removed. The extent of the ACTH release was estimated by the rate of steroid formation of the two adrenals.

ACTH reserve of the anterior pituitary was tested with purified lysine vasopressin (300 IU/mg) or with a CRF preparation obtained from the rat median eminence of hypophysectomized rats which had been operated on 1 day before. The fresh tissue was ground with 0,2 ml of 0,1 N HCL, the homogenate was centrifuged for 10 minutes at 3000 r. p. m. and the supernatant was adjusted to pH 7 by adding 0,1 N NaOH.

The corticotropin-releasing effect of these substances in rats with lesions of the stalk was studied. Intact rats anaesthetized with intraperitoneal injection of 4 mg/100 g body weight of nembutal served as controls. The animals were submitted to anaesthesia half an hour before the examination. The preparations containing the CRF were injected into the lateral tail vein and 15 minutes later the animals were killed by decapitation to assess the steroid production of the adrenals.

Adrenal sensitivity to ACTH in rats with lesions was measured using ACTH A_1 peptide which was administered intravenously via one of the lateral tail veins. Animals were decapitated 15 minutes after injection to assess corticosteroid production in vitro. Adrenal sensitivity to ACTH of rats with lesions was compared to that of animals hypophysectomized two hr. previously.

Simultaneously with the preparation of the hypothalamus extract the effective substance of the anterior pituitary was extracted as described above to determine its ACTH activity. 0,25 and 0,50 mg of the tissue of

the anterior lobe of intact rats and of rats bearing stalk lesions was administered as extract iv to animals hypophysectomized two hours earlier. The extent of the increase of the corticoidogenesis was determined 15 minutes after the injection and was considered as the index of the ACTH activity of the pars distalis. The results were expressed in microg total corticosteroids/100 mg adrenal/hr.

The ADH activity of the median eminence was measured in rats anaesthetized with ethanol by the method of DE WIED (1960).

The results are demonstrated in Figs. and a Table.

The effect of stress on intact rats and on rats with stalk lesions was studied to assess the steroid production. The results are presented in Fig. 41. It may be seen on the Fig. That exposure to a strange environment,

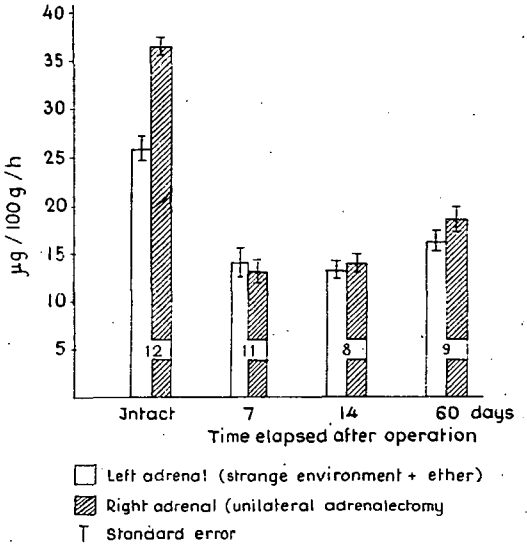


Fig. 41. Effect of stress on corticosteroid production (in vitro) of adrenal slices of intact rats and of rats with pituitary stalk lesion

and unilateral adrenalectomy caused a marked rise in corticosteroid production which showed an increasing tendency in the controls. In contrast, in rats with lesions in the stalk operated 7 or 14 days before neither stimuli caused any considerable change. The values of the animals submitted to stalk lesion 60 days before were slightly higher, however, there was no significant difference between the steroid production of the two adrenals.

Fig. 42. summarizes the results obtained following lysine vasopressin administration. The Fig. shows that corticosteroid production of adrenal slices of intact rats under nembutal anaesthesia rose linearly with the ADH doses. The data of rats bearing lesions in the stalk operated 1, 5, 7 and 14 days previously were similar. In such animals smaller amounts of vasopressin did not induce enhanced steroidogenesis, however, following a high dose (270 mU) a definite rise could be observed. In rats 60 days after lesion the effect of the 90 and 270 mU doses of lysine vasopressin

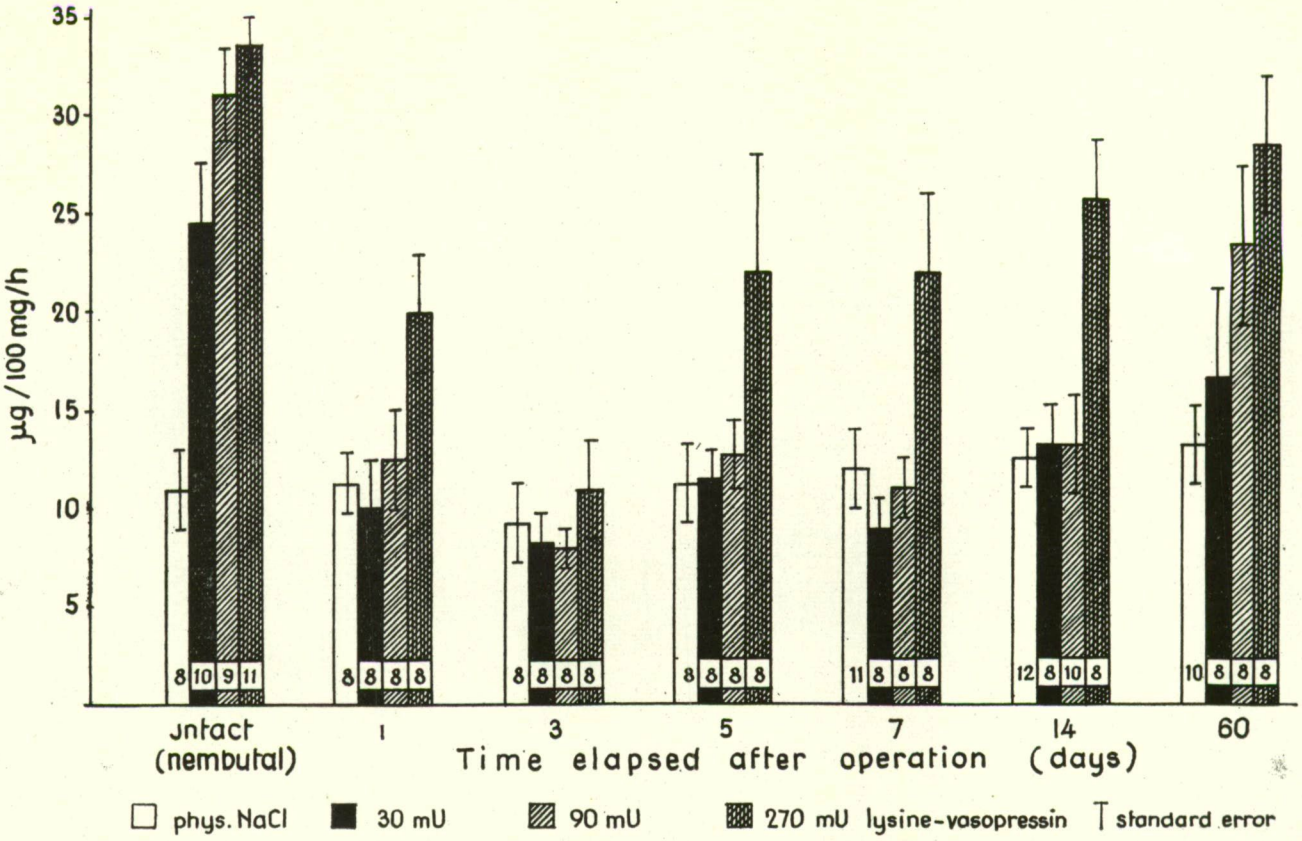


Fig. 42. Effect of lysine-vasopressin on corticosteroid production (in vitro) of adrenal slices of intact rats under nembutal anaesthesia and of rats with pituitary stalk lesion

was also significant. It seemed remarkable that in the oliguric interphase on the third postoperative day the lysine vasopressin was completely ineffective, the ACTH-adrenocortical axis of rats with pituitary stalk lesions was not sensitive to ADH.

The extract of the median eminence containing CRF proved also to be ineffective in animals with stalk lesion placed 3 days previously (Fig. 43.), whereas in the intact rats treated with nembutal the extract increa-

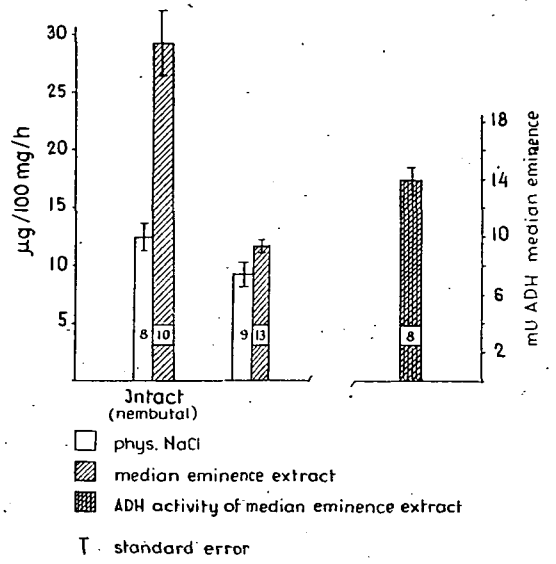


Fig. 43. Effect of median eminence extract of hypophyectomized rats on corticosteroid production (in vitro) of adrenal slices of intact rats and of rats with pituitary stalk lesion operated 3 days previously

sed corticoid production to more than the double. The activity of the ADH solution administered was 13,9 ± 0,9 mU, corresponding to the ADH content of each median eminence.

In the next experimental series the question was analysed why the ACTH-adrenocortical system of animals operated three days before failed to be sensitive to different stimuli. First of all the question arose whether the diminished response of the adrenal cortex might play a role in the development of the phenomenon. Therefore, we examined how ACTH affected the corticoid production in rats submitted to destruction of the stalk three days previously. The results were compared with those obtained in animals hypophysectomized two hours earlier, and with those of rats in which the stalk lesions had been placed 14 days before.

The data are depicted on Fig. 44. It is well visible that the adrenal function both in hypophysectomized rats and in those bearing lesions of the stalk changed linearly depending on the ACTH dose applied. Whereas the response of the adrenal cortex of animals with lesions of the stalk performed 3 days previously was in good agreement with that of the hypophysectomized group, the steroid production of rats submitted to stalk lesions 14 days before was definitely higher than following removal

of the pituitary. It should be noted that the activity of the ACTH preparation used in the latter experiments was higher than that of the one applied in the former; this is also shown by the difference between the

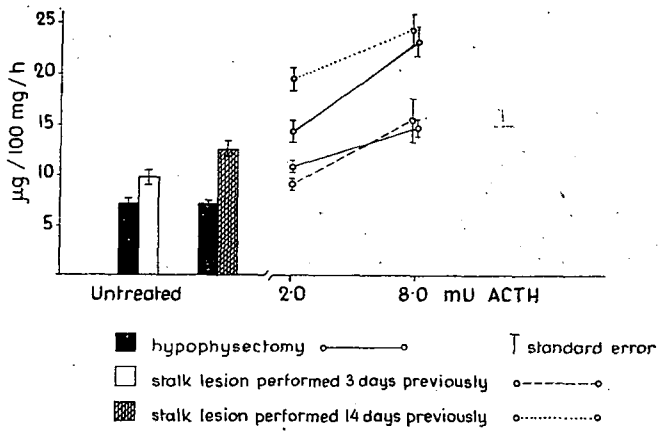


Fig. 44. Effect of ACTH (A_1 peptide) on corticosteroid production (in vitro) of adrenal slices of hypophysectomized rats and rats with pituitary stalk lesion

2 hypophysectomized groups. In any case the experiments revealed that the adrenal sensitivity of the animals with stalk lesions persisted, the in vitro corticosteroid production of the rats responded well to ACTH.

Finally, it seemed necessary to study the question whether after the stalk lesion the pituitary possessed an adequate active ACTH reserve sufficient to stimulate the adrenal cortex. In Chapter II. it has been described that after electrolytic lesion of the stalk the blood supply of the pituitary diminished, extensive necrosis developed; it may therefore be assumed that the hormone content of the gland changed, as well.

Table 32. gives an account of these observations. The Table demonstrates that after operation the weight of the anterior pituitary lobe decreased gradually, only the animals in which the pituitary stalk was destroyed 2 months previously showed a rising tendency, if, however, the body weight was also taken into consideration, the dependence on the elapse of time also held good in this case. The ACTH activity of the extracts of the anterior lobe was expressed as the rate of the in vitro total corticosteroid production (microg/h) related to 100 mg of the adrenal of rats hypophysectomized 2 hours earlier. If two different doses of pituitary extracts were used it could be established that the differences were roughly parallel. The hormone activity of the anterior lobe of rats bearing stalk lesion gradually declined from the first until the fifth day, the extract of the pars distalis of animals with five days-old lesions exerted the lowest activity. Subsequently a rise could again be detected and the values of the rats submitted to destruction of the stalk 60 days earlier approached those of the controls. Hence the animals bearing lesions of the stalk had a significant ACTH reserve and recent experiments also indicated that the cause of the disturbance in the function of the ACTH-

Table 32.

Effect of adenohyopphysial extract of normal rats and of rats with stalk lesion on total corticoid production (in vitro) of adrenal slices of hypophysectomized rats

	Time elapsed after operation	No. of animals	Body weihgt g	Weight of the anterior lobe of the pituitary g	Amount of the anterior lobe/rat 0,25 mg / 0,50 mg Total corticoid ug/100 mg/h	
I.	normal	10	203±1,7*	6,9±0,2	18,1±0,4	26,1±0,9
II.	1	11	206±1,7	5,8±0,1	16,9±1,5	23,5±1,9
III.	3	10	210±2,8	5,2±0,2	16,0±1,3	21,1±1,0
IV.	5	10	203±4,0	4,7±0,2	11,4±0,4	19,5±1,3
V.	7	9	216±4,5	4,5±0,2	13,5±0,8	21,4±1,3
VI.	60	8	276±18,3	5,0±0,3	15,7±0,7	24,8±1,2
		Probability:	I/II.	$p<0,001$	$p>0,05$	$p>0,05$
			I/III.	$p<0,001$	$p>0,05$	$p<0,01$
			I/IV.	$p<0,001$	$p<0,001$	$p<0,001$
			I/V.	$p<0,001$	$p<0,001$	$p<0,01$
			I/IV.	$p<0,001$	$p<0,01$	$p>0,05$

* Standard error

adrenocortical axis was in the first place due to the inhibition of the ACTH secretion.

In the previons subchapter it has been demonstrated that in the oliguric interphase associated with an increase of ADH discharge, ACTH mobilisation did not increase. On studying the cause of this phenomenon as a result of the experiments it seems possible to answer most of the questions raised.

Concerning the presumable differences of the effects of endogenous and exogenous ADH it could be established that neither the great amount of vasopressin released in the antidiuretic phase nor the administration of a large dose of extrinsic ADH augmented the corticoid production. Thus, there cannot be a substantial difference between endogenous and exogenous adiuretin.

The examination also revealed that the decreased ACTH content of the anterior pituitary cannot play a decisive role in the development of the phenomenon, as the anterior lobe possessed a considerable ACTH supply in all periods of the disturbances of the water metabolism occurring after destruction of the stalk.

The experiments described showed unequivocally that the sensitivity to ADH of the anterior pituitary of rats with stalk lesions had decreased and the steroid production only increased following administration of large quantities of ADH; and that in the oliguric phase, on the third postoperative day, administration of lysine vasopressin was completely ineffective. Although, the animals had a considerable ACTH reserve in this period, too, following the different effects (stress, administration of ADH and hypothalamus extracts) the inhibition of ACTH mobilization reached its maximum. It could further be observed that after ACTH administration the adrenal response of rats with three-days-old lesions was in complete harmony with that of the hypophysectomized animals. According to the data it may be assumed that in this period the adrenal function of animals bearing stalk lesions was similar to that of the hypophysectomized rats, and it is well known that neither ADH nor CRF affect hypophysectomized animals. (Lipscomb et al. 1960; Hearn et al. 1961; De Wied, 1961 a, b; Vernicos—Danellis, 1964).

Whether some kind of inhibitory substance is released together with ADH, has in the stage of our current experiments not yet been elucidated.

In the literature the question of identity of ADH and CRF is still an undecided problem.

Several data have been mentioned above suggesting that under certain conditions ADH has properties corresponding to CRF; this fact is also indicated by our own investigations. In addition it is also known that the CRF activity of the hypothalamus and neurohypophysial extracts cannot be completely explained by their ADH content (Royce and Sawyers, 1958, 1960; Gros and De Garilhe, 1959; Guillemin et al. 1959; 1960; McCann and Haberland, 1959; Rumsfeld and Porter, 1962). Most authors agree that the hypothalamus contains besides ADH several other polypeptides which exert in spite of their low vasopressor and antidiuretic effect a considerable CRF activity in vitro (De Garilhe, et al. 1960; Kappeler and Schwyzer, 1960; Li et al. 1961; Doepfner et al. 1963). In

our experiments extracts of the median eminence increased significantly the corticoid production in rats under nembutal anaesthesia, this change is, however, far more considerable than the similar one due to the ADH quantities found in the extracts prepared form the corresponding portion of the hypothalamus.

Finally, we dealt with the question by which mode of action the inhibition of ACTH mobilization affects rats with stalk lesions.

According to HARRIS (1951 a) the hypothalamus regulates the secretion of the anterior pituitary hormones via the humoral route; he assumes that chemical mediator substances exerting a hypophyseotrop effect form in the hypothalamus and the reaching the glandular cells of the anterior pituitary through the portal vessels influence hormone mobilization (so-called neurohumoral regulation). This hypothesis is supported by several observations. It has been shown that the interruption of the direct vascular connections between the hypothalamus and anterior pituitary (severance and destruction of the portal vessels, transplantation of the anterior pituitary) results in a hypofunction of the anterior pituitary (HARRIS, 1955 a; KOVÁCS, 1956, 1963; DÁVID et al. 1960). Other authors extracted substances exerting a hypophyseotropic effect from the hypothalamus, the region of the median eminence and the blood of the portal vessels (GUILLEMIN, 1955; SAFRAN and SCHALLY, 1955; PORTER and RUMSFIELD, 1956, 1959; GUILLEMIN et al. 1957, 1962). The direct hypothalamo-hypophysial vascular connection was also disrupted in our experimental rats as a consequence of the destruction of the pituitary stalk. However, the isolation of the anterior pituitary proved to be only temporary as owing to the regeneration of the portal vessels in agreement with the results reported by HARRIS (1950, 1955a) vascular connection between the hypothalamus and the anterior pituitary was again restored. In spite of this ACTH secretion did not increase and the normal function of the hypophysial-adrenocortical axis — as our results proved — was not restored. According to our own investigations the importance of the portal vessels regarding the influence of the anterior pituitary function cannot be denied, yet the conclusion had to be drawn that the influencing activity of the hypothalamus did not only come into play via the portal vessels and that in the induction of the hypofunction of the hypophysial-adrenocortical system other mechanisms must also be taken into account.

The decrease in the volume of the pars distalis cannot either be the only causative factor in the development of pituitary hypofunction, observations of partially hypophysectomized animals showed that the removal of 50 per cent of the anterior pituitary did not result in symptoms pointing to any significant endocrine deficiency (SMITH, 1932, GANONG and HUME, 1956; CAMPBELL, 1959); according to our own investigations following stalk lesion necrosis developed in about 50 per cent of the anterior lobe and in the surviving glandular tissue the circulation remained normal.

On the other hand, it might also be assumed that the function of the anterior pituitary also depends on the quality of the blood supplied by the portal vessels. Destruction of the stalk may inflict persistant damage to the hypothalamic structures producing the neurohumoral mediator

substance (CRF) and though it is possible for the mediator substances to reach the glandular cells of the anterior pituitary through the vessels, this after all does not happen as the mediator substances are produced in inadequate amounts. Accordingly, from the standpoint of pituitary function not only the quantity, but also the quality of the blood flowing to the anterior pituitary is an important factor. If the blood does not contain the needed amount of mediator substance in spite of an adequate blood flow hypopituitarism might ensue. The observation that in the oliguric phase the neurohypophysial mediator substances injected into the systemic circulation of rats with stalk lesions did not induce ACTH release, contradicted these comments. There are, however, also data suggesting that the „releasing factors" could not exert their effect adequately by the route of the systemic circulation (GREER et al. 1966).

Concerning the pituitary circulation the possibility was discussed above that following destruction of the stalk the surviving pituitary tissue did not get their blood supply through the portal vessels. If this assumption is correct this means that the anterior pituitary tissue can be divided into two portions; glandular cells the blood supply of which is provided by the hypothalamus through the portal vessels, and glandular portions not receiving their blood supply from the hypothalamus. As a result of the lesion of the stalk actually the necrosis developed in the central part supplied by the portal vessels and only the border which did not get its blood flow from the hypothalamus survived. In this case, on the other hand, according to the assumptions of ADAMS et al. (1964) the hypofunction of the pituitary developing after the destruction could also be explained by the fact that the portion of the anterior pituitary which was sensitive to CRF of hypothalamic origin perished, and the „releasing factors" were vainly still further produced in the hypothalamus and could reach the anterior pituitary through the now already regenerated portal vessels, this would be in vain considering that there — owing to the fact that the glandular cells of the anterior pituitary cannot regenerate — they would find instead of glandular cells sensitive to mediator substances, a scarred connective tissue.

Chapter VII

CONCLUSIONS

Recently interest has focussed in neuroendocrine research work on the hypothalamo- hypophysial system. Numerous data prove the importance of the hypothalamus and pituitary gland in the regulation of water metabolism. The hypothalamo-neurohypophysial and the hypothalamo-adenohypophysial systems markedly influence fluid and electrolyte homeostasis by several regulatory mechanisms. Many investigators studied these problems, however, numerous questions are not yet clear.

The purpose of the present work was to analyse in detail the role of the connection between the hypothalamus and pituitary in water metabolism. The experiments were performed on rats, the pituitary stalk was destroyed electrolytically by means of a stereotaxic apparatus. Subsequently the morphological alterations in the hypothalamo-hypophysial system, the changes in the water metabolism, as well as in the function of the ACTH-adrenocortical axis were studied by means of different methods.

The results and conclusions can be summarized as follows:

Following destruction of the pituitary stalk significant changes could be observed in the morphological picture of the hypothalamo-hypophysial system, in the water metabolism as well as in the function of the ACTH-adrenocortical axis.

After the lesion of the stalk extensive ischaemic necrosis developed in the anterior lobe, later fibrosis and atrophy was found in the necrotic area. The volume of the intermediate lobe did not change appreciably, whereas the posterior lobe gradually atrophied to a great extent.

Pituitary circulation was studied by means of India ink method as well as Sapirstein's ^{86}Rb isotope technique. It was established that following the destruction of the stalk the vascular connection between the hypothalamus, and the pituitary was interrupted, however, after a few weeks owing to the regeneration of the portal vessels the vascular connection was restored. It was remarkable that the blood flow of the surviving anterior lobe did not decrease immediately after the operation or either later. According to the result obtained the possibility cannot be ruled out that the blood supply of the pars distalis does not only originate from the long portal vessels.

The examinations also furnished data concerning the problem of the regeneration of the anterior pituitary. On this subject various investigators have reached contradictory conclusions. In the course of our experiments

an enlargement of the volume of the surviving part of the anterior lobe could not be observed in any case. Hence it is reasonable to assume that the glandular cells of the adenohypophysis are not capable of regeneration despite an adequate adenohypophysial blood supply and the restoration of the portal vascular connections.

The disturbance of the water metabolism developing after the lesion of the stalk was analysed by different methods. It was demonstrated that on applying oral water loads to rats with lesions of the stalk and on measuring the spontaneous water consumption and urinary output the disorder of water metabolism following destruction of the stalk had three phases. First water intake and urinary output increased then an interphase associated with oliguria and diminished water consumption lasting for 1—2 days ensued. Water retention was again followed by intensive polyuria and polydipsia.

A study of the cause of the development of the oliguric interphase indicated that this well reproducible phenomenon was induced by the antidiuretic hormone released in large amounts from the hypothalamo-neurohypophysial system damaged as a results of the destruction of the pituitary stalk. This conclusion was confirmed by the following data: Water retention only occurred in animals in which the lesion of the stalk could be demonstrated. In the oliguric period apart from an unchanged creatinine output specific gravity, sodium, potassium and chloride concentrations of the urine increased and the Gomori positive neurosecretory material disappeared from the posterior pituitary and hypothalamus; furthermore urinary ADH excretion was enhanced and the ADH content of the hypothalamo-neurohypophysial system diminished. By administration of ADH preparations and transplantation of the neurohypophysis an interphase-like state could be induced in animals suffering from diabetes insipidus. Reduction of the antidiuretic activity of the hypothalamo-neurohypophysial system (deprivation of water in the preoperative period, neurohypophysectomy following destruction of the stalk) diminished significantly, or suspended completely, the oliguric phase.

In the transitory period of polyuria preceding the interphase urinary ADH excretion did not increase significantly following administration of hypertonic NaCl solution. This observation indicated that the water retention lasting for 1—2 days was the result of a pathologic secretion of ADH released from the antidiuretic hormone centres damaged by the surgical manipulation. According to this theory ADH discharge during the interphase was not connected with normal regulatory mechanisms.

Following oral water loads urinary output gradually decreased in the majority of rats with stalk lesion and 1—2 months after the operation the volume of urine did not, or hardly, exceeded the average values of the non-operated controls. It seemed that the diabetes insipidus regressed. However, the normalization of the water metabolism was only virtual as the spontaneous water intake of the animals was still enhanced and the specific gravity and electrolyte content of the urine was below normal. Following cortisone treatment the diuretic reaction increased and if the rats were loaded orally with an isotonic solution of NaCl instead of tap water, polyuria developed.

On the basis of the results obtained the question arises which factors are involved in the amelioration of the disturbance of water metabolism.

The investigations suggest that the cessation of the polyuric reaction can be correlated with the reduction of the glomerular filtration rate. On the other hand, it is also possible that in rats submitted to destruction of the pituitary stalk the production of the antidiuretic hormone is restored and this might also play a role in the diminution of the polyuria. This assumption is supported by the fact that following lesion of the stalk in rats in which the diuretic response returned to normal, the ADH content of the hypothalamo-neurohypophysial system and the urinary ADH excretion were higher, and by hyperosmosis a more pronounced ADH mobilization could be induced than in the persistently polyuric animals.

After partial destruction of the pituitary stalk certain changes could also be observed in the structure of the pituitary, and in the water metabolism of the rats. In the anterior lobe on the site of the lesion a focal ischaemic necrosis developed; in this area later fibrosis and atrophy could be seen. It was surprising that the atrophy of the posterior pituitary was also intensive following partial destruction of the stalk. In these animals the impairment of the antidiuretic hormone system could also be demonstrated by functional methods. An oliguric interphase associated with excessive ADH release developed, the spontaneous water consumption and the diuretic reaction following isotonic saline loads were enhanced. On the other hand, after water loads the diuretic reaction and urinary ADH excretion were normal, thus the functional disturbance was only slight.

Following adrenalectomy the quantity of the urine decreased. In rats adrenalectomized previously destruction of the pituitary stalk did not result in polyuria. Hence, corticosteroids markedly influence water homeostatis. Therefore, on studying the relation between the hypothalamo-hypophysial system and water metabolism in rats with stalk lesion it seemed advisable to extend the experiments to the analysis of the function of the adrenal cortex.

The function of the ACTH-adrenocortical axis was estimated by different methods. The changes observed after the application of various procedures were not always unequivocal. It could, however be established that following lesion of the stalk the function of the adrenal cortex decreased which may be chiefly explained by the inhibition of ACTH-mobilization. However, the different partial functions of the adrenal cortex did not respond in the same way; when corticosterone production decreased, aldosterone production remained unchanged.

For the further elucidation of the function of the ACTH-adrenocortical system, the rats bearing stalk lesions were submitted to cortisone and oestrogen hormone treatment. Cortisone induces adrenocortical atrophy. whereas administration of oestrogen hormone causes enlargement of the adrenal cortex. These alterations, occur also in rats with pituitary stalk lesions, however, only to a smaller extent. The results suggest that the presence of an intact hypothalamo-hypophysial system is not necessary for the effect of cortisone and oestrogen hormone exerted on the adrenal cortex.

It is known that the antidiuretic hormone induces under various experimental conditions ACTH mobilization. As during the oliguric interphase antidiuretic hormone is released in great amounts from the hypothalamo-neurohypophysial system damaged owing to the destruction of the stalk, it seemed worth while to examine the function of the pituitary-adrenocortical axis in rats with stalk lesions in the oliguric period. The conclusion can obviously be drawn that in the course of the interphase there is no close parallelism between the extent of the secretion of ADH and the activity of the hypophysial-adrenocortical axis; as in spite of the enhanced endogenous ADH mobilization in these animals plasma corticosterone and in vitro corticosteroid production decreased.

In the previous subchapter it has been demonstrated that in the interphase the sensitivity of the adrenals to ACTH was not diminished. It was also demonstrated that the oliguric animals though having a considerable ACTH reserve are not capable of reacting with ACTH mobilisation following various stimuli (stress, large doses of exogenous ADH, administration of hypothalamic extract containing CRF). The fact that the release of ACTH is inhibited in rats with the lesion of the stalk may be a decisive factor why ADH mobilized during the interphase does not exert an effect on the secretion of ACTH and thus on the function of the adrenal cortex.

The cause of the inhibition of the release of ACTH in rats with stalk lesions is not known. The explanation of Harris seems the most probable. He states that for the function of the anterior pituitary the intactness of the anterior pituitary, the intactness of the portal vascular system is necessary. This possibility is, however, contradicted by the fact that after the regeneration of the portal vessels in rats with lesion of the stalk the function of the hypophysial-adrenocortical axis does not become normal. According to the results obtained the following possibilities can be taken into account: after the lesion of the stalk owing to the irreversible injury of the hypothalamic structures CRF is not produced in suitable amounts. It may, however, also be that as a result of the impairment of the hypophysial stalk, the central part of the anterior lobe supplied by the long portal vessels which is sensitive to CRF becomes necrotic. Thus the releasing factors reach in vain the anterior pituitary through the regenerated portal vessels, ACTH mobilisation cannot ensue because the glandular cells capable of responding to the mediator substances are destroyed by the operation and are replaced by scar tissue, inactive from the hormonal aspect.

The investigations have furnished further data that hypothalamus and pituitary form a close anatomic and functional unit. It is therefore justified to accept the existence of the hypothalamo-hypophysial system. The hypothalamo- neurohypophysial and the hypothalamo-adenohypophysial systems influence considerably water metabolism and the secretion of corticosteroids. There is a complex interaction between the functions of the two systems exerting effects on different levels. The normal function of the hypothalamus and the pituitary gland is a prerequisite for the maintenance of fluid and electrolyte homeostasis.

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